

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
23 August 2001 (23.08.2001)

PCT

(10) International Publication Number  
**WO 01/61015 A2**

(51) International Patent Classification?: C12N 15/54,  
15/62, 15/70, 9/12, 1/21, C12Q 1/68, C12P 19/34

(21) International Application Number: PCT/EP01/01790

(22) International Filing Date: 16 February 2001 (16.02.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
09/506,153 17 February 2000 (17.02.2000) US

(63) Related by continuation (CON) or continuation-in-part  
(CIP) to earlier application:  
US 09/506,153 (CIP)  
Filed on 17 February 2000 (17.02.2000)

(71) Applicant (for all designated States except US): QIAGEN  
GMBH [DE/DE]; Max-Volmer-Strasse 4, 40724 Hilden  
(DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): LÖFFERT, Dirk  
[DE/DE]; Bahlenstrasse 8, 40589 Düsseldorf (DE). MIS-  
SEL, Andreas [DE/DE]; Kirchfeldstrasse 93, 40215 Düs-  
seldorf (DE). KANG, Jie [DE/DE]; Meisenweg 25, 40822  
Mettmann (DE).

(74) Common Representative: QIAGEN GMBH;  
Max-Volmer-Strasse 4, 40724 Hilden (DE).

(81) Designated States (national): JP, US.

(84) Designated States (regional): European patent (AT, BE,  
CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC,  
NL, PT, SE, TR).

**Published:**

— without international search report and to be republished  
upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: THERMOSTABLE CHIMERIC NUCLEIC ACID POLYMERASES AND USES THEREOF

(57) Abstract: Novel thermostable chimeric nucleic acid polymerases and methods for their generation and use are disclosed. It is shown that these chimeric nucleic acid polymerases, such as DNA polymerases, can be constructed using enzymatically active domains, isolated from different proteins or chemically synthesized. It is demonstrated that chimeric nucleic acid polymerases of the present invention possess the chemical and physical properties of their component domains (e.g., exonuclease activity, thermostability) and that the chimeric polymerases are thermostable.



**WO 01/61015 A2**

## THERMOSTABLE CHIMERIC NUCLEIC ACID POLYMERASES AND USES THEREOF

### Cross Reference to Related Applications

This application claims the benefit of U.S. Nonprovisional Application No. 09/506,153, filed 17 February 2000.

### Field of the Invention

The present invention is in the field of molecular biology. The present invention is directed to novel thermostable chimeric enzymes useful for the generation of nucleic acids, methods for making thermostable chimeric nucleic acid polymerases, and methods useful for polymerizing nucleic acids using a thermostable chimeric nucleic acid polymerase. Specifically, the invention is directed to chimeric thermostable DNA polymerases and their uses.

### Background of the Invention

Nucleic acid polymerases are an important class of compounds that enzymatically link (polymerize) nucleotides to form larger polynucleotide chains (e.g., DNA or RNA strands). Nucleic acid polymerases typically utilize a template polynucleotide (in either a single-strand or double-strand form) for nucleic acid synthesis, as in conventional nucleic acid replication, transcription, or reverse transcription. Other nucleic acid polymerases, e.g., terminal transferase (TdT), are capable of *de novo* polymerization, that is, template independent nucleic acid synthesis.

All known nucleic acid polymerases possess an enzymatic domain that catalyzes the formation of a phosphodiester bond between two nucleotides, utilizing the 5' carbon triphosphate of one nucleotide and the 3' carbon hydroxyl group of another nucleotide. Nucleic acid polymerases synthesize nascent polynucleotides by linking the 5' phosphate of one nucleotide to the 3' OH group of the growing polynucleotide strand. This process is known and commonly referred to by persons skilled in the art as 5'-3' polymerization.

In addition, nucleic acid polymerases possess a wide range of ancillary chemical properties useful for nucleic acid synthesis. These properties include, but are not limited to:

- product and/or template specificity (e.g., RNA or DNA);
- single-strand or double-strand template specificity;

- processivity - a measure of the ability of a nucleic acid polymerase to generate a nascent polynucleotide from a template polynucleotide without dissociating from the template;
- 5 • extension rate - a measure of the rate at which nucleotides are added to a growing polynucleotide strand;
- fidelity - a measure of the accuracy (or conversely the error rate) with which a nucleic acid polymerase synthesizes a polynucleotide complementary to a template polynucleotide;
- 10 • nick translation - the ability of a nucleic acid polymerase to degrade the preceding nucleotide strand of a double strand molecule simultaneous to polymerizing a nascent strand;
- proofreading - the ability of a nucleic acid polymerase to remove an incorrectly linked nucleotide from a polynucleotide before further polymerization occurs; and
- 15 • thermostability - the ability of a nucleic acid polymerase to retain activity after exposure to elevated temperatures.

Many of these properties are the result of one or more discrete functional domains within a polymerase holoenzyme. Three extensively studied enzymatically active domains of nucleic acid polymerase include: a 5'-3' polymerase domain, responsible for  
20 polynucleotide synthesis; a 5'-3' exonuclease domain, responsible for polynucleotide digestion of the 5' end of a polynucleotide, useful for nick translation; and a 3'-5' exonuclease domain, responsible for polynucleotide digestion of the 3' end of a polynucleotide, allowing for proofreading, and thus improving the fidelity of the polymerase. Some studies indicate that selection, incorporation, and extension of the  
25 correct nucleotide, versus an incorrect nucleotide, is a variable property of the 5'-3' polymerase domain, thus affecting polymerase fidelity in concert with proofreading activity (Mendelman et al., 1990; Petruska et al., 1988).

DNA polymerases can be categorized into six families based on amino acid homology. These families consist of; pol I, pol  $\alpha$ , SONDZEICHEN pol  $\beta$ , SONDZEICHEN  
30 DNA-dependent RNA polymerase, reverse transcriptase, and RNA-dependent RNA polymerase (Joyce and Steitz, 1994). Table 1 summarizes the enzymatic features of a few representative DNA polymerases.

**Table 1. DNA polymerase enzymatic activity**

DNA polymerase	(N terminus ----- C terminus)			Thermo-stability	de novo polymerase
	5'-3' exonuclease	3'-5' exonuclease	5'-3' polymerase		
<i>E. coli</i> pol I	(+)	(+)	(+)	(-)	(-)
Klenow fragment	(-)	(+)	(+)	(-)	(-)
<i>E. coli</i> pol II	(-)	(+)	(+)	(-)	(-)
<i>E. coli</i> pol III	(+)	(+)	(+)	(-)	(-)
T4 pol	(-)	(+)	(+)	(-)	(-)
T7 pol	(-)	(+)	(+)	(-)	(-)
M-MuLV RT	(-)	(-)	(+)	(-)	(-)
TdT	(-)	(-)	(+)	(-)	(+)
<i>Taq</i> pol	(+)	(-)	(+)	(+)	(-)
Stoffel fragment	(-)	(-)	(+)	(+)	(-)
<i>Tbr</i> pol	(+)	(-)	(+)	(+)	(-)
<i>Tli</i> pol	(-)	(+)	(+)	(+)	(-)
<i>Tma</i> pol	(-)	(+)	(+)	(+)	(-)
<i>Tth</i> pol	(+)	(-)	(+)	(+)	(-)
<i>Pfu</i> pol	(-)	(+)	(+)	(+)	(-)
<i>Psp</i> pol	(-)	(+)	(+)	(+)	(-)
<i>Pwo</i> pol	(-)	(+)	(+)	(+)	(-)

Because of the diversity of properties and characteristics potentially exhibited by nucleic acid polymerases generally, practitioners in the art have sought to modify, to alter, or to recombine various features of nucleic acid polymerases in an effort to develop new and useful variants of the enzyme. Initially, polymerase truncations and deletions were developed. The Klenow fragment, for example, was the first nucleic acid polymerase variant developed. Klenow fragments exist as a large C-terminal truncation of DNA polymerase I (pol I), possessing an enzymatically active 3'-5' exonuclease and 5'-3' polymerase domains, but lacking altogether the 5'-3' exonuclease domain of native pol I (Klenow and Henningsen, 1970; Jacobson et al., 1974; and Joyce and Grindley, 1983).

Since the advent of the polymerase chain reaction (PCR) methodology (including derivative methodologies such as reverse transcription PCR, or RT-PCR), resilient nucleic acid polymerases, capable of withstanding temperature spikes as high as 95°C without a subsequent significant loss in enzymatic activity (i.e., thermostable) have become vital tools in modern molecular biology. The use of thermostable enzymes to amplify nucleic acid sequences is described in U.S. Pat. Nos. 4,683,195 and 4,683,202. A thermostable DNA polymerase from *Thermus aquaticus* (*Taq*) has been cloned, expressed and purified from recombinant cells (Lawyer et al., 1989; U.S. Pat. Nos. 4,889,818 and 5,079,352. PCR is also described in many U.S. patents, including U.S. Pat. Nos. 4,965,188, 4,683,195, 4,683,202, 4,800,159, 4,965,188, 4,889,818, 5,075,216, 5,079,352, 5,104,792, 5,023,171, 5,091,310, and 5,066,584.).

As depicted in Table I, *Taq* DNA polymerase possesses enzymatically active 5'-3' polymerase and 5'-3' exonuclease domains, but it exhibits only background levels of 3'-5' exonuclease activity (Lawyer et al., 1989; Bernard et al., 1989; Longley et al., 1990). Crystallographic data revealed that *Taq* polymerase contains a 3'-5' exonuclease domain (Eom et al., 1996); comparisons of the crystal structure of the Klenow fragment from *Bacillus* DNA polymerase I, *Taq* DNA polymerase, and *E. coli* DNA polymerase I have shown, however, that critical residues required to carry out a 3'-5' exonuclease activity are missing in the 3'-5' exonuclease domain of *Taq* DNA polymerase (Kiefer et al., 1997). Park et al. (1997), have determined that *Taq* DNA polymerase possesses none of three sequence motifs (*Exo* I, II, and III) within the 3'-5' exonuclease domain and necessary for 3'-5' exonuclease activity. Because *Taq* polymerase exhibits essentially no 3'-5' exonuclease activity (i.e., proofreading capability), the error rate of *Taq* DNA polymerase is high compared to other DNA polymerases that possess an enzymatically active 3'-5' exonuclease domain (Flaman et al., 1994). The *Taq* DNA polymerase structure thus comprises a 5'-3' exonuclease domain occurring at the N-terminal region of the polypeptide (residues 1-291), followed by an enzymatically inactive 3'-5' exonuclease domain (residues 292-423), and a C-terminal 5'-3' polymerase domain (Park et al., 1997).

Since *Taq* DNA polymerase does not possess an enzymatically active 3'-5' exonuclease domain, providing a proofreading feature to the polymerase, the use of *Taq* DNA polymerase becomes less desirable for most nucleic acid amplification applications, e.g., for PCR sequencing protocols or amplification for protein expression, which require complete identity of replication products to the template nucleic acid. Depending on the phase of PCR during which an error becomes incorporated into the PCR product (e.g., in an early replication cycle), the entire population of amplified DNA could contain one or more sequence errors, giving rise to a nonfunctional and/or mutant gene product. Nucleic acid polymerases that possess an enzymatically active 3'-5' exonuclease domain (i.e., proofreading activity), therefore, are especially preferred for replication procedures requiring high fidelity.

Due to the scientific and commercial importance of PCR in modern molecular biology, the reliance of PCR protocols on nucleic acid polymerases of particular characteristics, and in view of the enzymatic deficiencies of *Taq* polymerase, an enormous amount of research and development has focussed on developing new and useful thermostable DNA polymerase variants and/or assemblages.

One approach has been directed to the discovery and isolation of new thermophilic nucleic acid polymerases, which may possess a unique and/or improved collection of catalytic properties. As a result, thermostable nucleic acid polymerases have been isolated from a variety of biological sources, including, but not limited to, species of the

taxonomic genera, *Thermus*, *Thermococcus*, *Thermotoga*, *Pyrococcus*, and *Sulfolobus*. These polymerases possess a variety of chemical characteristics, as illustrated in Table 1. Some of these naturally occurring thermostable DNA polymerases possess enzymatically active 3'-5' exonuclease domains, providing a natural proofreading capability and, thus, exhibiting higher fidelity than *Taq* DNA polymerase. Naturally occurring proofreading thermostable polymerases include: *Pfu* polymerase (isolated from *Pyrococcus furiosus*), *Pwo* polymerase (isolated from *Pyrococcus woesei*), *Tli* polymerase (isolated from *Thermococcus litoralis*), and *Psp* polymerase (isolated from *Pyrococcus* sp. GB-D). All of these naturally occurring thermostable polymerases are commercially available (*Tli* polymerase and *Psp* polymerase are marketed as Vent<sup>®</sup> and Deep Vent<sup>SONDZEICHEN®</sup> DNA polymerase, respectively, by New England Biolabs, Beverly, MA). These DNA polymerases show slower DNA extension rates and an overall lower processivity when compared to *Taq* DNA polymerase, however, thus rendering these naturally occurring thermostable DNA polymerases less desirable for PCR, despite their higher fidelity.

In an effort to compensate for the deficiencies of individual thermostable polymerases, a second approach has been to develop multiple enzyme assemblages, combining, for example, *Taq* polymerase and a proofreading enzyme, such as *Pfu* polymerase or Vent<sup>SONDZEICHEN®</sup> polymerase. These multiple-enzyme mixtures exhibit higher PCR efficiency and reduced error rates when compared to *Taq* polymerase alone (Barnes, 1994). Mixtures of multiple thermostable enzymes are commercially available (e.g., the Failsafe<sup>™</sup> PCR system from Epicentre, Madison, WI). PCR protocols utilizing multiple polymerase mixtures are still prone to error, however, and require the practitioner to perform preliminary experimental trials, to determine special optimized solution conditions necessary for multiple-enzyme reaction mixtures.

A third approach has been to develop new and useful variants of *Taq* polymerase through deletion/truncation techniques. The Stoffel fragment, for example, is a 544 amino acid C-terminal truncation of *Taq* DNA polymerase, possessing an enzymatically active 5'-3' polymerase domain but lacking 3'-5' exonuclease and 5'-3' exonuclease activity. Other commercially available thermostable polymerase deletions include Vent<sup>SONDZEICHEN®</sup> (exo<sup>-</sup>) and Deep Vent<sup>SONDZEICHEN®</sup> (exo<sup>-</sup>) (New England Biolabs, Beverly, MA). Deletion mutations serve only to remove functional domains of a nucleic acid polymerase, however, and do not add any novel features or enzymatic properties.

Polymerase mutagenesis is yet another approach that has been attempted to develop new and useful nucleic acid polymerase variants. Park et al. (1997) performed site-directed mutagenesis of 4 amino acids in the enzymatically inactive 3'-5' exonuclease domain of *Taq* polymerase in an effort to activate the proofreading ability of this domain.

The resultant mutant exhibited an increase of exonuclease activity over that of naturally occurring *Taq* polymerase. The reported increase was a mere two-fold increase above background exonuclease activity, however; an insignificant rise in exonuclease activity that is unlikely to increase PCR fidelity.

5 Bedford et al. (1997) developed a recombinant mesophilic DNA pol I from *E. coli*. They succeeded to insert a thioredoxin binding domain from T7 DNA polymerase into *E. coli* pol I. The inserted 76 amino acid binding domain improved polymerase binding to a template polynucleotide, thus increasing the processivity of the recombinant *E. coli* pol I but did not improve or provide any novel enzymatic activity to the polymerase.

10 Recently Gelfand et al. (1999) combined fusion protein technology with mutagenesis to eliminate or substantially reduce 5'-3' exonuclease activity and 3'-5' exonuclease activity in recombinant polymerases. Once again, no improved or additional enzymatic activity was provided by the fusion polymerase.

Frey et al. (1999) attempted to engineer chimeric polymerases utilizing  
15 enzymatically active domains from *Taq*, *Tne*, and *E. coli* DNA polymerases. Although they successfully substituted the non-functional 3'-5' exonuclease domain of *Taq* DNA polymerase with a functional 3'-5' exonuclease domain from another DNA polymerase, their resultant chimeric polymerase lost significant, if not all, enzymatic activity after only one minute at 80°C or 95°C (i.e., they are not thermostable), and thus are not useful for  
20 performing PCR protocols without the successive addition of fresh polymerase for each cycle.

Despite these intense research efforts, there remains a need in the art for thermostable nucleic acid polymerases that possess improved or novel assemblages of enzymatically active domains. Despite its enzymatic deficiencies, *Taq* DNA polymerase  
25 remains the most widely used enzyme for processing *in vitro* amplification of nucleic acids. In particular, there has been long felt need for a nucleic acid polymerase possessing the 5'-3' polymerization qualities of *Taq* polymerase, but which also possesses 3'-5' exonuclease (proofreading) activity.

### 30 Summary of the Invention

In response to the long felt need for new and useful nucleic acid polymerases, a novel approach for producing thermostable nucleic acid polymerases was invented. The present invention represents the first thermostable chimeric nucleic acid polymerase, useful for continuous PCR protocols, obtained by combining at least two enzymatically  
35 active domains from different proteins by means of recombinant DNA techniques.

The present invention is directed to novel thermostable chimeric enzymes useful for the generation of nucleic acids, methods for making thermostable chimeric nucleic acid

polymerases, and methods useful for polymerizing nucleic acids using a thermostable chimeric nucleic acid polymerase. The thermostable chimeric nucleic acid polymerase of the present invention comprises at least two enzymatically active domains, which are non-naturally associated. The recombinant association of the enzymatically active domains results in a composite enzyme not found in nature. The thermostable chimeric nucleic acid polymerase of the present invention possesses new or improved catalytic properties compared to nucleic acid polymerases known in the art.

The thermostable chimeric nucleic acid polymerase of the present invention offers several advantages over previous approaches to develop novel nucleic acid polymerases. The present invention provides a single enzyme that possesses a suite of chemical properties, the combination of which may not necessarily exist in nature, but nonetheless is useful in molecular biology. The chimeric nucleic acid polymerase of the present invention eliminates the need to specifically develop multiple-enzyme reaction mixtures, which are often difficult to optimize and expensive to use, and the necessity to add successive amounts of fresh enzyme during each cycle of a PCR program. The invention thus facilitates the rapid, efficient, and accurate generation of nucleic acid molecules, particularly in regard to PCR protocols.

#### Definitions

As used herein, an "enzymatically active domain" refers to any polypeptide, naturally occurring or synthetically produced, capable of mediating, facilitating, or otherwise regulating a chemical reaction, without, itself, being permanently modified, altered, or destroyed. Binding sites (or domains), in which a polypeptide does not catalyze a chemical reaction, but merely forms noncovalent bonds with another molecule, are not enzymatically active domains as defined herein. In addition, catalytically active domains, in which the protein possessing the catalytic domain is modified, altered, or destroyed, are not enzymatically active domains as defined herein. Enzymatically active domains, therefore, are distinguishable from other (nonenzymatic) catalytic domains known in the art (e.g., detectable tags, signal peptides, allosteric domains, etc.).

As defined herein, a 3'-5' exonuclease domain refers to any polypeptide capable of enzymatically cleaving a nucleotide from the 3' end of a di- or polynucleotide, a 5'-3' exonuclease domain refers to any polypeptide capable of enzymatically cleaving a nucleotide from the 5' end of a di- or polynucleotide, and a 5'-3' polymerase domain refers to any polypeptide capable of enzymatically linking the 5' phosphate of one nucleotide to the 3' OH group of another nucleotide.

Polypeptide domains that are "non-naturally associated", refer to specific polypeptides that are not naturally produced within a single polypeptide; that is, the



polypeptide domains are not naturally translated from a common nucleic acid transcript in a naturally occurring organism. Non-naturally associated polypeptide domains include domains isolated from functionally distinct proteins, separately produced by an organism of one or more species, or synthetically generated, as well as polypeptide domains  
5 isolated from functionally similar proteins, but naturally produced by organisms of different species, or synthetically generated. The term "non-naturally associated polypeptide domains" refers to domains that are associated or fused only through human intervention; the term expressly excludes naturally occurring enzymes or fragments thereof.

As used herein, the term "chimeric protein" encompasses all proteins that contain  
10 two or more polypeptide domains that are non-naturally associated (regardless of whether the domains are naturally produced by organisms of the same species, different species, or synthetically generated). A chimeric nucleic acid polymerase of the present invention must necessarily possess two or more non-naturally associated domains, as defined herein.

15 The term "thermostable" generally refers to the resilience of a substance to relatively high temperature treatment. A thermostable enzyme is an enzyme that retains its definitive enzymatic activity despite exposure to relatively high temperature. A thermostable nucleic acid polymerase, as generally understood by practitioners in the art and as defined herein, refers to a polymerase that is useful for PCR protocols; i.e., not  
20 requiring successive or supplemental addition of enzyme after each high temperature step of the PCR program cycle. The chimeric nucleic acid polymerase of the present invention is thermostable, in that it is useful for PCR protocols, because it does not require successive or supplemental addition of polymerase after each high temperature step of the PCR program cycle.

25 A preferred thermostable chimeric polymerase of the present invention is one that allows a thermal polymerase chain reaction to proceed with only an initial supply of polymerase at the start of the PCR program. Preferably, a thermostable chimeric nucleic acid polymerase retains some measurable enzymatic activity at its normal operating temperature (typically about 72°C) after exposure to 95°C for three minutes. More  
30 preferably, a thermostable chimeric nucleic acid polymerase is able to withstand one minute at 95°C without significant loss (> 5% loss) in enzymatic activity. In other words, a preferred thermostable chimeric nucleic acid polymerase retains at least about 95% of its polymerase activity at its normal operating temperature (typically about 72°C) after one minute at 95°C. Even more preferably, a thermostable chimeric nucleic acid polymerase  
35 is able to withstand three minutes at 95°C without significant loss in enzymatic activity. A most preferred thermostable chimeric nucleic acid polymerase is able to withstand ten minutes at 90°C and still retain at least about 50% of its enzymatic activity at its normal

operating temperature. In other words, the polymerase displays a "half life" (the length of time it takes for a substance to lose one half of its initial activity) of ten minutes at 90°C. Ideally, a thermostable chimeric nucleic acid polymerase displays a half-life comparable to the half-life measurement of naturally occurring thermostable nucleic acid polymerases.

- 5 For example a most desirable thermostable chimeric nucleic acid polymerase displays a half-life at 90°C comparable to that of *Taq* polymerase, approximately 90 minutes.

The present invention is directed generally to all thermostable chimeric nucleic acid polymerases comprising at least two non-naturally associated enzymatically active domains. As defined herein, a nucleic acid polymerase is any enzyme that catalyzes the  
10 formation of chemical bonds between (chemically bonds) nucleotides to form polynucleotide chains, that is, any enzyme that promotes nucleic acid polymerization. The thermostable chimeric nucleic acid polymerases of the present invention include all types of nucleic acid polymerases, without limitation to product or template specificity, molecular requirements, or chemical properties (e.g., RNA vs. DNA, single strand vs. double strand,  
15 high fidelity, etc.).

One embodiment of the present invention is directed to a thermostable chimeric DNA polymerase, preferably a chimeric DNA polymerase wherein the enzymatically active domains are isolated from naturally occurring proteins from two or more species, or any mutants, variants, or derivatives thereof.

- 20 As used herein, mutant, variant, and derivative polypeptides refer to all chemical permutations of a given polypeptide, which may exist or be produced, that still retain the characteristic molecular activity that is definitive of that polypeptide.

The thermostable chimeric nucleic acid polymerase of the present invention is unexpected in view of the fact that enzymatically active domains may be isolated from a  
25 wide variety of sources, yet still retain their enzymatic activities (e.g., polymerase, exonuclease) and chemical properties (e.g., thermostability, processivity). Enzymatically active domains isolated from organisms of different taxonomic kingdoms and from completely different families of proteins may be fused to produce an entirely novel, yet functional, nucleic acid polymerase. For example, enzymatically active domains from a  
30 eubacterium polymerase of e.g., *Taq* polymerase may be chimerically joined with enzymatically active domains from an archaeon polymerase (e.g., *Pwo*, *Sso*, and *Pho* polymerases).

- Retention of thermal stability in a fusion protein engineered from different thermophilic proteins is highly unexpected. Attempts to construct chimeric polymerases  
35 have failed to produce thermostable chimeric polymerases (see Frey et al., 1999). The underlying principles of thermal stability of proteins derived from thermophilic organisms are not known. Even small changes in the amino acid sequence of thermoresistant

proteins result in a significant decrease in thermal stability and an associated reduction in enzymatic activity of the protein. Maintenance of, or an increase in, thermal stability of thermostable DNA polymerase has only been accomplished by truncation of a DNA polymerase (e.g., Barnes, 1995). The present invention represents the first chimeric nucleic acid polymerase, containing enzymatically active domains from different thermostable proteins, that possess thermostable properties.

In a preferred embodiment, at least one of the enzymatically active domains of the chimeric nucleic acid polymerase is isolated from a DNA polymerase produced by a thermophilic organism, preferably an organism of a genus selected from the group of genera consisting of: *Thermus*, *Thermococcus*, *Thermotoga*, *Pyrococcus*, *Pyrodictium*, *Bacillus*, *Sulfolobus*, and *Methanobacterium*. Most preferably, at least one of the enzymatically active domains of the chimeric nucleic acid polymerase is isolated from a DNA polymerase selected from the group consisting of: *Thermoplasma acidophilum* (*Tac*) polymerase; *Thermus aquaticus* (*Taq*) polymerase; *Thermococcus barossii* (*Tba*) polymerase; *Thermus Brockianus* (*Tbr*) polymerase; *Tfi* polymerase; *Thermus flavus* (*Tfl*) polymerase; *Thermococcus litoralis* (*Tli*) polymerase; *Thermococcus pacificus* (*Tpac*) polymerase; *Thermus ruber* (*Tru*) polymerase; *Thermus thermophilus* (*Tth*) polymerase; *Pyrodictium abyssi* (*Pab*) polymerase; *Pyrococcus furiosus* (*Pfu*) polymerase; *Pyrococcus hellenicus* (*Phe*) polymerase; *Pyrococcus horikoshii* (*Pho*) polymerase; *Pyrococcus kodakarensis* (*Pko*) polymerase; *Pyrococcus* sp. strain KOD1 (KOD) polymerase; *Pyrococcus* sp. strain ES4 (ES4) polymerase; *Pyrodictium occultum* (*Poc*) polymerase; *Pyrococcus* sp. GB-D (*Psp*) polymerase; *Pyrococcus woesei* (*Pwo*) polymerase; *Thermotoga maritima* (*Tma*) polymerase; *Thermotoga neapolitana* (*Tne*) polymerase; *Bacillus sterothermophilus* (*Bst*) polymerase; *Sulfolobus acidocaldarius* (*Sac*) polymerase; *Sulfolobus solfataricus* (*Sso*) polymerase; *Methanobacterium thermoautotrophicum* (*Mth*) polymerase; and mutants, variants, and derivatives thereof.

In another embodiment of the invention, the enzymatically active domains are selected from the group consisting of: 5'-3' exonuclease domain, 3'-5' exonuclease domain, and 5'-3' polymerase domain. Preferably the enzymatically active domains are naturally occurring domains, isolated from two or more species, most preferably the enzymatically active domains are isolated from naturally occurring thermostable proteins, mutants, variants, or derivatives thereof.

Another aspect of the present invention relates to an isolated polynucleotide encoding a thermostable chimeric nucleic acid polymerase comprising at least two non-naturally associated enzymatically active domains. Preferably the enzymatically active domains are isolated from different species.

A related aspect of the invention is directed to a method for synthesizing a recombinant nucleic acid that encodes a thermostable chimeric nucleic acid polymerase comprising at least two non-naturally associated enzymatically active domains.

5 A further aspect of the invention relates to a vector comprising a polynucleotide that encodes a thermostable chimeric nucleic acid polymerase having at least two non-naturally associated enzymatically active domains. Preferred vectors are expression vectors, which will be suitable for production of the encoded chimeric nucleic acid polymerase in transformed host cells.

10 Another aspect of the invention includes a recombinant host cell transformed with a vector comprising a polynucleotide that encodes a thermostable chimeric nucleic acid polymerase possessing at least two non-naturally associated enzymatically active domains.

15 A related aspect of the invention is directed to a method for producing a thermostable chimeric nucleic acid polymerase comprising at least two non-naturally associated enzymatically active domains.

Another aspect of the invention is directed to a process of nucleic acid polymerization, which necessarily utilizes a thermostable chimeric nucleic acid polymerase having at least two non-naturally associated enzymatically active domains.

20 A related aspect of the invention is directed to a kit useful for polymerization of nucleic acid, comprising a thermostable chimeric nucleic acid polymerase having at least two non-naturally associated enzymatically active domains. Preferably, the kit further comprises at least one reagent suitable for nucleic acid polymerization. Most preferably, the kit further comprises at least one reagent selected from the group consisting of one or more additional enzymes, one or more oligonucleotide primers, a nucleic acid template,  
25 any one or more nucleotide bases, an appropriate buffering agent, a salt, or other additives useful in nucleic acid polymerization.

#### Brief Description of the Drawings

30 **Fig. 1** is a photograph of an ethidium bromide (EtdBr)-stained agarose gel, which depicts the polymerase activity of thermostable chimeric DNA polymerases using a primer extension reaction. Lane 1 shows a nucleic acid ladder, used as a gel reference marker. Lanes 2, 6 and 10 show negative controls (without addition of polymerase). Lane 3, 4, 5 show the activity of 0.05, 0.03 and 0.01 units *Taq* DNA polymerase, respectively. Lanes 7-9 illustrate polymerase activity of undiluted cleared lysate, a 1:1, and 1:5 diluted cleared  
35 lysate, of a *Pho/Taq* chimeric polymerase, respectively.

**Fig. 2** is a photograph of an ethidium bromide (EtdBr)-stained agarose gel, which depicts the thermostability of a thermostable chimeric DNA polymerase compared to *Taq*

DNA polymerase, using a primer extension reaction. DNA polymerases were incubated for various time spans at 90°C and assayed for remaining polymerase activity. Lanes 1 and 11 show a nucleic acid ladder, used as a gel reference marker. Lanes 2, 10, 12, and 20 represent negative control reactions (without addition of polymerase). Lanes 3-9 and lanes 13-19 illustrate DNA polymerase activity after incubation of *Taq* DNA polymerase and a *Pho/Taq* chimeric DNA polymerase at 90°C for 0, 10, 15, 30, 60, 90, and 120 min, respectively.

**Fig. 3** is a photograph of an ethidium bromide (EtdBr)-stained agarose gel, which depicts 3'-5' exonuclease activity of three different thermostable DNA polymerases. **(A)** illustrates PCR product using a wild type primer combination. **(B)** illustrates PCR product using a mutant primer pair. Lane 1 is a nucleic acid ladder, used as a gel reference marker. The PCR amplification product of *Taq* DNA polymerase is shown in lanes 2-5; *Pfu* DNA polymerase I PCR product is shown in lanes 6-9; and a *Pho/Taq* thermostable chimeric DNA polymerase PCR product is shown in lanes 10-13. Duplicate side-by-side reactions are shown representing undigested (the first and third lane for each enzyme used), and digested (the second and fourth lane for each enzyme used) PCR product.

**Fig. 4** is a photograph of an ethidium bromide (EtdBr)-stained agarose gel, which illustrates the combined effect of primer extension efficiency and polymerase processivity on PCR efficiency of three different thermostable DNA polymerases. The photograph illustrates PCR products obtained in duplicate reactions using different primer extension times. **(A)** indicates PCR products obtained with *Taq* DNA polymerase. **(B)** illustrates PCR products obtained with a *Pho/Taq* thermostable chimeric DNA polymerase. **(C)** shows PCR products generated with *Pfu* DNA polymerase I. Lane 1 is a nucleic acid ladder, used as a gel reference marker. Lanes 2-3 show PCR products amplified after primer extension for 1 min. Lanes 4-5 show PCR products amplified after primer extension for 30 sec. Lanes 6-7 show PCR products amplified after primer extension for 10 sec. Lanes 8-9 show PCR products amplified after primer extension for 5 sec.

#### Detailed Description of the Invention

Genetic engineering techniques were successfully employed to generate the first thermostable chimeric nucleic acid polymerase, containing enzymatically active domains, not naturally found within a single protein. The chimeric nucleic acid polymerase and methods described herein encompass all thermostable nucleic acid polymerases, without limitation to product or template specificity, molecular requirements, or chemical properties. For example, chimeric nucleic acid polymerases of the present invention include single or double strand DNA polymerases, RNA polymerases, and reverse transcriptases. Thermostable chimeric nucleic acid polymerases of the present invention

may possess any number and/or combination of properties and features including, but not limited to, template dependence or independence, high processivity, high fidelity, proofreading, nick translation, and high extension rates. Persons skilled in the art will understand and appreciate that these features are due, in large part, to the presence and characteristics of discrete polypeptide domains within the holoenzyme. Essential to the chimeric nucleic acid polymerase of the present invention is that it possess at least two enzymatically active domains that are not naturally associated, and the chimeric nucleic acid is thermostable.

Enzymatically active domains may be isolated from any natural polypeptide, or may be synthetically produced. Natural polypeptides include any polypeptide found in nature, and from any organism of any taxonomic group. Enzymatically active domains useful in the present invention also include variant, mutant, or derivative forms of domains found in nature. Enzymatically active domains further include domains that may not be found in nature, e.g., polypeptides randomly generated or engineered in the laboratory or selected from a non-naturally generated library of polypeptides. For the purposes of this invention, enzymatically active domains need only necessarily possess an enzymatic activity that is functional within the chimeric nucleic acid polymerase of the invention. The thermostable chimeric nucleic acid polymerases of the present invention specifically contemplates incorporation into a nucleic acid polymerase, enzymatically active domains that are absent, inactive, or weakly active in the naturally occurring protein.

Persons skilled in the art will know and appreciate that a wide variety of enzymatic domains exist that perform the same or similar enzymatic functions. For example, DNA polymerases possess 3'-5' exonuclease domains of a wide range of enzymatic functionality; from little or no 3'-5' exonuclease activity (as seen in *Taq* polymerase), to fully functional 3'-5' exonuclease activity (as seen in *E. coli* pol I), to thermostable 3'-5' exonuclease activity (as seen in *Pwo* polymerase). It is understood by practitioners in the art that enzymatically active domains of individual polymerases are considered separate and distinct enzymatically active domains, as defined herein. Thus, the incorporation of an enzymatically active domain from one polymerase into a second polymerase produces, by definition, a chimeric polymerase, regardless of whether the second polymerase naturally possesses its own enzymatically active domain of similar functionality.

Preferably, genetic engineering techniques may be used to generate novel thermostable DNA polymerases possessing either 5'-3' polymerase activity and 3'-5' exonuclease activity; or 5'-3' polymerase activity, 3'-5' exonuclease activity and 5'-3' exonuclease activity derived from different thermostable DNA polymerases, e.g. *Taq* polymerase, *Pho* polymerase, *Pwo* polymerase, *Sso* polymerase, and *Tpac* polymerase.

Preferred thermostable chimeric nucleic acid polymerases of the present invention include a 5'-3' polymerase domain of *Taq* polymerase. For example, the Stoffel fragment is a 544 residue N-terminal deletion of *Taq* polymerase possessing an enzymatically active 5'-3' polymerase domain and an enzymatically inactive 3'-5' exonuclease domain.

5 Generally, a *Taq* 5'-3' polymerase domain is at least about 544 residues in length, and includes any mutant, variant, or derivative of the Stoffel fragment of *Taq* polymerase, as defined herein. A 552 amino acid polypeptide, residue numbers 281-832 of *Taq* polymerase (SEQ ID NO:1), is an especially preferred enzymatically active *Taq* 5'-3' polymerase domain useful in the present invention.

10 Alternatively, the thermostable chimeric nucleic acid polymerases of the present invention may include a 5'-3' polymerase domain of *Tth* polymerase. *Tth* polymerase is capable of reverse transcription. Thermostable chimeric nucleic acid polymerases, which include the *Tth* 5'-3' polymerase domain, therefore, may be used for reverse transcription reactions (e.g., RT-PCR). Preferably, the 5'-3' polymerase domain of *Tth* polymerase is  
15 about 562 residues in length, including residue numbers 273-834 of *Tth* polymerase (SEQ ID NO:2), and includes any mutant, variant, or derivative thereof.

Preferred thermostable chimeric nucleic acid polymerases of the present invention also include an enzymatically active 3'-5' exonuclease domain of a thermostable polymerase. Preferred 3'-5' exonuclease domains include the enzymatically active 3'-5' exonuclease domains of *Pho* polymerase, *Pwo* polymerase, *Sso* polymerase, and *Tpac*  
20 polymerase. Most preferred are residues 1-396 of *Pho* polymerase (SEQ ID NO:3), residues 1-396 of *Pwo* polymerase (SEQ ID NO:4), residues 1-421 of *Pwo* polymerase (SEQ ID NO:5), residues 1-508 of *Sso* polymerase (SEQ ID NO:6), residues 1-395 of *Tpac* polymerase (SEQ ID NO:16), and any mutants, variants, or derivatives of any one of  
25 these 3'-5' exonuclease domains, as defined herein.

A process for synthesizing a recombinant nucleic acid encoding a thermostable chimeric nucleic acid polymerase of the invention necessarily comprises isolating at least two nucleic acid fragments each encoding at least one enzymatically active domain, which is not naturally associated with the other enzymatically active domain (i.e., derived from  
30 separate polypeptides), and genetically combining the nucleic acids of the enzymatically active domains to form a chimeric nucleic acid.

For production of thermostable chimeric nucleic acid polymerases according to the invention, the nucleic acid encoding a chimeric nucleic acid polymerase may be stably inserted into a genetic vector, preferably the nucleic acid is operably inserted into an  
35 expression vector, and most preferably the vector construct is capable of replication within a host organism, such that the nucleic acid encoding a thermostable chimeric nucleic acid polymerase is capable of being transcribed and translated into a polypeptide. A preferred

mode of making the chimeric nucleic acid polymerase of the present invention includes culturing a host cell containing a nucleic acid encoding a thermostable chimeric nucleic acid polymerase under conditions suitable for expression of the chimeric nucleic acid polymerase by the host cell, and isolating the chimeric nucleic acid polymerase expressed  
5 from said cell culture.

Methods for generating recombinant nucleic acids, vector construction, host cell transformation, and polypeptide expression systems useful in the practice of this invention can involve a wide variety of modern genetic engineering techniques, tools, and biological sources that are well known in the art and routinely practiced by those skilled in the art.  
10 Exemplary techniques and methods are described in detail herein by way of preferred example, but are not limiting to the practice of the invention. The present invention incorporates by reference in their entirety techniques and supplies well known in the field of molecular biology, including, but not limited to, techniques and supplies described in the following publications:

- 15 Ausubel, F.M. et al. eds., Short Protocols In Molecular Biology (4th Ed. 1999) John Wiley & Sons, NY. (ISBN 0-471-32938-X).
- Freshney, R.I. Culture of Animal Cells (1987) Alan R. Liss, Inc.
- Old, R.W. & S.B. Primrose, Principles of Gene Manipulation: An Introduction To Genetic Engineering (3d Ed. 1985) Blackwell  
20 Scientific Publications, Boston. Studies in Microbiology; V.2:409 pp. (ISBN 0-632-01318-4).
- Sambrook, J. et al. eds., Molecular Cloning: A Laboratory Manual (2d Ed. 1989) Cold Spring Harbor Laboratory Press, NY. Vols. 1-3. (ISBN 0-87969-309-6).
- 25 Winnacker, E.L. From Genes To Clones: Introduction To Gene Technology (1987) VCH Publishers, NY (translated by Horst Ibelgauf). 634 pp. (ISBN 0-89573-614-4).

The thermostable chimeric nucleic acid polymerases described herein are  
30 especially useful for generating a desired target nucleic acid. Thermostable chimeric nucleic acid polymerases of the invention, having at least two enzymatically active domains that are not naturally associated may be utilized under conditions sufficient to allow polymerization of a nascent nucleic acid. Generally, this method includes any method of nucleic acid generation, replication, amplification, transcription, or reverse  
35 transcription known in the art that utilizes a conventional nucleic acid polymerase, wherein the nucleic acid polymerase is substituted or combined with a chimeric nucleic acid polymerase of the present invention. Preferably the method of amplification is polymerase chain reaction, utilizing a thermostable chimeric nucleic acid polymerase. PCR is



described herein as an exemplary protocol capable of utilizing the compositions and methods of the present invention without limitation. Persons skilled in the art will understand that the present invention has utility in other processes requiring the polymerization of nucleic acid (e.g., RT-PCR).

5 PCR is a technique well known in the art. PCR is used to amplify nucleic acids by subjecting a reaction mixture to cycles of: (i) nucleic acid denaturation, (ii) oligonucleotide primer annealization, and (iii) nucleic acid polymerization. Preferred reaction conditions for amplification comprise thermocycling, i.e., alternating the temperature of the reaction mixture to facilitate each of the steps of the PCR cycle. PCR is typically extended through  
10 multiple cycles of denaturation, annealization and replication, augmented (optionally and preferably) with an initial prolonged denaturation step and a final prolonged extension (polymerization) step. To perform the repetitive steps of thermocycling, it is preferable to employ an enzyme that is capable of tolerating exposure to relatively high temperature without a subsequent significant loss in enzyme activity; i.e., a thermostable enzyme. The  
15 use of a thermostable enzyme for PCR protocols permits the repetitive steps of increasing and decreasing reaction temperatures without the need to supplement, or otherwise add, enzyme after each successive high temperature step of the PCR program cycle.

Also included in the invention is a kit that includes a thermostable chimeric nucleic acid polymerase and one or more additional reagents suitable for nucleic acid  
20 polymerization reactions. Such components may include, but are not limited to: one or more additional enzymes, one or more oligonucleotide primers, a nucleic acid template, any one or more nucleotide bases, an appropriate buffering agent, a salt, or other additives useful in nucleic acid polymerization.

Additional enzymes of the kit include any enzyme that may be used in combination  
25 with the thermostable chimeric nucleic acid polymerase of the invention. For example, multiple-polymerase kits are known in the art. Numerous polymerases are known and commercially available to persons skilled in the art, and include DNA polymerases, RNA polymerases, and reverse transcriptases (commercial suppliers include: Roche Diagnostics., Indianapolis, IN; Life Technologies, Inc., Rockville, MD; New England  
30 Biolabs, Inc., Beverly, MA; Perkin Elmer Corp., Norwalk, CT; Pharmacia LKB Biotechnology, Inc., Piscataway, NJ; Qiagen, Inc., Valencia, CA; Stratagene, La Jolla, CA).

Oligonucleotide primers useful in the present invention may be any oligonucleotide of two or more nucleotides in length. Preferably, PCR primers are about 15 to about 30  
35 bases in length and are not palindromic (self-complementary) or complementary to other primers that may be used in the reaction mixture. Primers may be, but are not limited to, random primers, homopolymers, or primers specific to a target oligonucleotide template

(e.g., a sequence specific primer). Oligonucleotide primers are oligonucleotides used to hybridize to a region of a target nucleic acid to facilitate the polymerization of a complementary nucleic acid. In PCR protocols, primers serve to facilitate polymerization of a first nucleic acid molecule complementary to a portion of an oligonucleotide template, and also to facilitate replication of the oligonucleotide. Any primer may be synthesized by a practitioner of ordinary skill in the art or may be ordered and purchased from any of a number of commercial vendors (e.g., from Roche Diagnostics, Indianapolis, IN; Life Technologies, Inc., Rockville, MD ; New England Biolabs, Inc., Beverly, MA; Pharmacia LKB Biotechnology, Inc., Piscataway, NJ). It will be understood that a vast array of primers may be useful in the present invention, including those not specifically disclosed herein, without departing from the scope or preferred embodiments thereof.

A nucleic acid template is defined as any polynucleotide molecule used to provide a nucleic acid sequence from which a polynucleotide complementary to the template may be generated. The synthesis of DNA from a DNA template may be accomplished according to the invention by utilizing a thermostable chimeric DNA polymerase. The synthesis of RNA from a DNA template may be accomplished according to the invention by utilizing a thermostable chimeric RNA polymerase. The synthesis of DNA from an RNA template may be accomplished according to the invention by utilizing a thermostable chimeric nucleic acid polymerase that exhibits reverse transcriptase activity.

Nucleotide bases useful in the present invention may be any nucleotide useful in the polymerization of a nucleic acid. Nucleotides may be naturally occurring, unusual, modified, derivative, or artificial. Nucleotides may be unlabeled, or detectably labeled by methods known in the art (e.g., using radioisotopes, vitamins, fluorescent or chemiluminescent moieties, digoxigenin). Preferably the nucleotides are deoxynucleoside triphosphates, dNTPs (e.g., dATP, dCTP, dGTP, dTTP, dITP, dUTP,  $\alpha$ -thioSONDZEICHEN-dNTPs, biotin-dUTP, fluorescein-dUTP, digoxigenin-dUTP, 7-deaza-dGTP). dNTPs are also well known in the art and are commercially available (e.g., from Roche Diagnostics, Indianapolis, IN; New England Biolabs, Inc., Beverly, MA; Pharmacia LKB Biotechnology, Inc., Piscataway, NJ).

Buffering agents and salts useful in the present invention provide appropriate stable pH and ionic conditions for nucleic acid synthesis. A wide variety of buffers and salt solutions and modified buffers are known in the art that may be useful in the present invention, including agents not specifically disclosed herein. Preferred buffering agents include, but are not limited to, TRIS, TRICINE, BIS-TRICINE, HEPES, MOPS, TES, TAPS, PIPES, CAPS. Preferred salt solutions include, but are not limited to solutions of; potassium chloride, potassium acetate, potassium sulfate, ammonium sulfate, ammonium chloride, ammonium acetate, magnesium chloride, magnesium acetate, magnesium

sulfate, manganese acetate, sodium chloride, sodium acetate, lithium chloride, and lithium acetate.

Other additives capable of facilitating nucleic acid generation and amplification, other than those disclosed for the first time by this invention, are known in the art. In accordance with the compositions and methods of this invention, one or more of these additives may be incorporated in a DNA/RNA polymerization kit according to the present invention to optimize the generation and replication of polynucleotides. Additives may be organic or inorganic compounds. Agents useful in the present invention include, but are not limited to, polypeptides such as phosphatase, human serum albumin, bovine serum albumin (BSA), ovalbumin, albumax, casein, gelatin, collagen, globulin, lysozyme, transferrin,  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, phosphorylase b, myosin, actin,  $\beta$ -galactosidase, lectins, *E. coli* single-stranded binding (SSB) protein, phage T4 gene 32 protein, and the like, or fragments or derivatives thereof. Examples of nonpolypeptide additives include, but are not limited to; homopolymeric nucleic acid, heteropolymeric nucleic acid, tRNA, rRNA, sulfur-containing compounds, acetate-containing compounds, dimethylsulfoxide (DMSO), glycerol, formamide, betain, tetramethylammonium chloride (TMAC), polyethylene glycol (PEG), Tween 20, NP 40, ectoine, and polyoles.

It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the compositions and methods of the invention described herein are obvious and may be made without departing from the scope of the invention or the embodiments disclosed herein. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting of the invention.

#### EXAMPLE 1: Construction of a Thermostable Chimeric DNA Polymerase Gene

Chimeric thermostable DNA polymerase constructs containing enzymatically active domains from different (source) thermostable DNA polymerases were generated using recombinant DNA techniques. The 3'-5' exonuclease domain of various thermostable polymerases were recombinantly linked to the 5'-3' polymerase domain of *Taq* polymerase or *Tth* polymerase. The particularly preferred enzymatic domains and domain borders, described herein in detail, were selected and tested as preferred embodiments, and are not to be considered limiting in scope of the thermostable chimeric nucleic acid polymerase of the invention, or the enzymatically active domains useful therein.

Appropriate microbial strains or genomic DNA preparations, from which the enzymatically active domains used in the construction of chimeric nucleic acid polymerase were isolated, were purchased from commercial suppliers, e.g., from DSMZ GmbH

(Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH), Braunschweig, Germany. Specifically chosen strains included *Thermus aquaticus* (order # DSM 625), *Thermus thermophilus* (order # DSM 579), *Pyrococcus furiosus* (order # DSM 3638), *Pyrococcus woesei* (order # DSM 3773), *Pyrococcus horikoshii* (order # DSM 3638),  
5 *Sulfolobus solfataricus* (order # DSM 5833), and *Thermococcus pacificus* (order # DSM 10394). A multiplicity of genomic DNA extraction, purification, and isolation techniques useful to obtain the desired enzymatically active domains are well known in the art.

Modified PCR amplification techniques and/or cloning procedures such as restriction digestion and ligation using appropriate enzymes were used to obtain the chimeric DNA  
10 polymerase constructs. Primers appropriate to amplify polynucleotides encoding particular enzymatic domains from the source thermostable DNA polymerases were synthesized according to the nucleotide sequences of the source thermostable DNA polymerase. DNA sequences of the source thermostable DNA polymerases are published in GenBank. The synthesis of oligonucleotide primers is well known to  
15 practitioners in the art, and may also be ordered from commercial oligonucleotide suppliers (e.g., Life Technologies, Gaithersburg, MD).

PCR primers were of special design. The primers contained a nucleotide sequence complementary to the terminal region of a particular enzymatic domain of interest within a source DNA polymerase. The primers also contained a noncomplementary nucleotide  
20 sequence region as well to provide; i) an appropriate restriction enzyme site, to facilitate genetic manipulation (e.g., vector insertion), or ii) sequence information (e.g., complementarity), to facilitate fusion to a second, non-naturally associated enzymatic domain. For example, primers designed to facilitate fusion of a 3'-5' exonuclease domain to a 5'-3' polymerase domain contained a sequence, one half of which was  
25 complementary to a terminal region of the 3'-5' exonuclease domain of interest (e.g., residues 388-396 of *Pho* polymerase) and one half of which was complementary to a terminal region of the 5'-3' polymerase domain (e.g., residues 281-288 of *Taq* polymerase).

As an initial step, various enzymatic domains were amplified by PCR. The PCR  
30 reaction mixture contained: 2.5 units of *Taq* polymerase (Qiagen, Valencia, CA) and 0.1 to 0.2 units of *Pfu* polymerase (Stratagene, La Jolla, CA); an appropriate amount of the specially designed primers, as described above (0.2 to 1.0  $\mu$ M); genomic DNA isolated from the appropriate microorganism containing the source thermostable polymerase; and 200  $\mu$ M of each dNTP in a 1x PCR buffer (Qiagen, Valencia, CA). A 3-step PCR cycling  
35 program was run, consisting of an initial denaturation step at 94° C, an annealing step and an extension step. The PCR ran for 25-35 cycles, depending upon the desired amount of product. The size of the PCR product was checked by agarose gel electrophoresis

against an appropriate DNA size marker. The correctly sized PCR product was gel-purified using the QIAquick™ Gel Extraction Kit (Qiagen, Valencia, CA).

Once isolation and amplification of the polynucleotides encoding the enzymatic domains chosen for chimeric polymerase construction were obtained, the component  
5 enzymatic domains were combined, in equivalent concentrations, in a composite PCR reaction, together with 2-5 units of *Pfu* polymerase (Stratagene, La Jolla, CA), and 200 μM of each dNTP in 1x PCR buffer (Qiagen, Valencia, CA). This PCR mixture did not contain any primer oligonucleotides. This reaction mixture was subjected to 10 to 15 PCR cycles.

10 During the composite PCR, the single strand polynucleotides encoding each of the enzymatically active domains hybridize at their respective terminal regions of complementarity (due to the specially designed primers as described above). The hybridized single strand polynucleotides encoding each of the enzymatically active domains form a single composite polynucleotide template, thus serving as primers for  
15 each other. *Pfu* polymerase extends the 3' terminal end of each of the enzymatically active domains, creating a single polynucleotide containing the chimeric DNA polymerase gene construct.

After the initial 10 to 15 cycles of chimeric DNA polymerase gene construction, oligonucleotide primers, appropriate to amplify the full-length chimeric DNA polymerase  
20 gene, were added to the PCR mixture. The PCR ran for 20-30 additional cycles, depending upon the desired amount of chimeric DNA polymerase PCR product. The size of the PCR product was checked by agarose gel electrophoresis and the correctly sized PCR product was gel-purified as described above.

The purified chimeric DNA polymerase gene was then subjected to restriction  
25 digestion with the appropriate restriction enzyme to cut the polynucleotide at restriction sites located at the terminal ends of the chimeric DNA polymerase gene. These sites were originally generated by the specially designed primers described above.

30 **EXAMPLE 1.1: Construction of a *Pho/Taq* Thermostable Chimeric DNA Polymerase Gene**

A polynucleotide encoding the enzymatically active 3'-5' exonuclease domain of *Pho* DNA polymerase was linked to a polynucleotide encoding the enzymatically active 5'-3' polymerase domain and the nonfunctional 3'-5' exonuclease domain of *Taq* DNA polymerase. A polynucleotide encoding amino acids 271-832 (SEQ ID NO:7) of *Taq* DNA  
35 polymerase was recombinantly linked to the 3' end of a polynucleotide encoding amino acids 1-396 (SEQ ID NO:3) of *Pho* DNA polymerase following the procedures detailed in

Example 1 above, producing a polynucleotide that encodes a novel *Pho/Taq* thermostable chimeric DNA polymerase (SEQ ID NO:8).

EXAMPLE 1.2: Construction of a *Pwo/Taq* Thermostable Chimeric DNA

5 Polymerase Gene

A polynucleotide encoding the enzymatically active 3'-5' exonuclease domain of *Pwo* DNA polymerase was linked to a polynucleotide encoding the enzymatically active 5'-3' polymerase domain of *Taq* DNA polymerase. A polynucleotide encoding amino acids 271-832 (SEQ ID NO:7) of *Taq* DNA polymerase was recombinantly linked to the 3' end of a polynucleotide encoding amino acids 1-396 (SEQ ID NO:4) of *Pwo* DNA polymerase following the procedures detailed in Example 1 above, producing a polynucleotide that encodes a novel *Pwo/Taq* thermostable chimeric DNA polymerase (SEQ ID NO:9).

EXAMPLE 1.3: Construction of a *Sso/Taq* Thermostable Chimeric DNA

15 Polymerase Gene

A polynucleotide encoding the enzymatically active 3'-5' exonuclease domain of *Sso* DNA polymerase was linked to a polynucleotide encoding the enzymatically active 5'-3' polymerase domain of *Taq* DNA polymerase. A polynucleotide encoding amino acids 281-832 (SEQ ID NO:1) of *Taq* DNA polymerase was recombinantly linked to the 3' end of a polynucleotide encoding amino acids 1-508 (SEQ ID NO:6) of *Sso* DNA polymerase following the procedures detailed in Example 1 above, producing a polynucleotide that encodes a novel *Sso/Taq* thermostable chimeric DNA polymerase (SEQ ID NO:10).

This chimeric construct, possessing a smaller *Taq* 5'-3' polymerase domain than that used in Examples 1.1 and 1.2, also demonstrates that specifically determined domain borders of an enzymatic domain are not essential to the invention. What is essential for the domain is that it retain its definitive enzymatic activity.

EXAMPLE 1.4: Construction of a *Tpac/Taq* Chimeric DNA Polymerase Gene

A polynucleotide encoding the enzymatically active 3'-5' exonuclease domain of *Tpac* DNA polymerase was linked to a polynucleotide encoding the enzymatically active 5'-3' polymerase domain of *Taq* DNA polymerase. A polynucleotide encoding amino acids 271-832 (SEQ ID NO:7) of *Taq* DNA polymerase was recombinantly linked to the 3' end of a polynucleotide encoding amino acids 1-395 (SEQ ID NO:16) of *Tpac* DNA polymerase following the procedures detailed in Example 1 above, producing a polynucleotide that encodes a novel *Tpac/Taq* chimeric DNA polymerase (SEQ ID NO:17).

EXAMPLE 1.5:        Construction of Variant Thermostable Chimeric DNA Polymerase Genes

To further demonstrate that a thermostable chimeric nucleic acid polymerase may be generated using an enzymatically active domain of varying domain borders (provided the enzymatic activity of the domain is retained), a *Pwo/Taq* chimeric DNA polymerase variant of the thermostable chimeric polymerase generated in Example 1.2 was constructed. This variant construct comprised a polynucleotide encoding amino acids 271-832 (SEQ ID NO:7) of *Taq* DNA polymerase recombinantly linked to the 3' end of a polynucleotide encoding amino acids 1-421 (SEQ ID NO:5) of *Pwo* DNA polymerase following the procedures detailed in Example 1 above, producing a polynucleotide that encodes a second novel *Pwo/Taq* thermostable chimeric DNA polymerase (SEQ ID NO:11).

EXAMPLE 1.6:        Construction of a *Pho/Tth* Thermostable Chimeric DNA Polymerase Gene

To demonstrate that a thermostable chimeric nucleic acid polymerase may be generated using an enzymatically active polymerase domain other than that of *Taq* polymerase, a polynucleotide encoding the enzymatically active 3'-5' exonuclease domain of *Pho* DNA polymerase was linked to a polynucleotide encoding the enzymatically active 5'-3' polymerase domain of *Tth* DNA polymerase. A polynucleotide encoding amino acids 273-834 (SEQ ID NO:2) of *Tth* DNA polymerase was recombinantly linked to the 3' end of a polynucleotide encoding amino acids 1-396 (SEQ ID NO:3) of *Pho* DNA polymerase following the procedures detailed in Example 1 above, producing a polynucleotide that encodes a novel *Pho/Tth* thermostable chimeric DNA polymerase (SEQ ID NO:12).

This chimeric construct, possessing a *Tth* 5'-3' polymerase domain that is also capable of reverse transcription activity, also demonstrates a thermostable chimeric nucleic acid polymerase of the present invention useful for RT-PCR protocols.

EXAMPLE 1.7:        Construction of a Thermostable Chimeric DNA Polymerase Gene Encoding More Than Two Enzymatically Active Domains

The chimeric nucleic acid polymerase gene of the invention may encode two or more enzymatically active domains, of which two more domains are non-naturally occurring. In addition the enzymatically active domains may be derived from any polypeptide source naturally occurring or synthetically produced.

For example, the practitioner may wish to construct a thermostable chimeric nucleic acid polymerase possessing both the 5'-3' polymerase domain and the 5'-3' exonuclease domain of *Taq* polymerase, as well as the 3'-5' exonuclease domain of another

polymerase (e.g., *Pho* polymerase). In this instance, a polynucleotide encoding the 5'-3' exonuclease domain of *Taq* polymerase (known to be contained within amino acids 1-291 of *Taq* polymerase) would be recombinantly linked to 5' end of a polynucleotide encoding the 3'-5' exonuclease domain of *Pho* polymerase (e.g., SEQ ID NO: 3) and the 5'-3' polymerase domain of *Taq* DNA polymerase (e.g., SEQ ID NOs: 1 or 7), which was earlier demonstrated in Examples 1.1 and 1.5.

**EXAMPLE 2: Construction of a Thermostable Chimeric DNA Polymerase Vector**

The isolated chimeric DNA polymerase genes of Examples 1.1 through 1.6 were each ligated into a vector, linearized using the appropriate restriction enzyme. Ligation was performed overnight at 16° C using T4 DNA ligase and an appropriate buffer (Life Technologies, Gaithersburg, MD) in a final volume of 20 µl.

**EXAMPLE 3: Construction of a Thermostable Chimeric DNA Polymerase Host Cell**

The ligated recombinant vectors of Example 2 were used to transform calcium-competent M15[pRep4] cells (Qiagen, Valencia, CA ) or DH5SONDZEICHENα competent cells. Aliquots of the transformation mixture were spread onto agar plates containing ampicillin and kanamycin (for M15[pRep4] cells), or ampicillin only (for DH5α competent cells), and incubated overnight at 37° C.

Colonies of successfully transformed cells were transferred to LB media containing the appropriate antibiotic selection, and incubated overnight. Plasmid isolation preparations were performed using QIAprep™ Spin Kit or Plasmid Midi Kit (both from Qiagen, Valencia, CA). Presence of the chimeric DNA polymerase gene was verified by restriction digest analysis and the chimeric DNA polymerase gene sequenced by techniques well known in the art.

The chimeric DNA polymerase genes were cloned into either pQE-30 or pQE-31 expression vectors (Qiagen, Valencia, CA) containing a six-histidine tag sequence preceding the respective DNA polymerase sequence.

**EXAMPLE 4: Expression and Purification of a Thermostable Chimeric DNA Polymerase**

Thermostable chimeric DNA polymerase gene expression of the successfully transformed host cells from Example 3, was induced by IPTG. Harvested cells were lysed by sonification and lysozyme treatment or a simple heat treatment. Chimeric His-tagged protein was purified in batch format using Ni-NTA agarose (Qiagen, Valencia, CA) following standard protocol procedures.



Eluates were ultrafiltrated using Nanosep<sup>SONDZEICHEN®</sup> ultrafiltration units (Pall Deutschland GmbH Holding, Dreieich, Germany). Alternatively, heat treated cleared lysate was centrifuged through Ultrafree filterunits 300.000 (Sigma, Deisenhofen, Germany), to remove contaminating nucleic acids, and was subsequently concentrated  
5 using Nanosep<sup>SONDZEICHEN®</sup> or Microsep<sup>SONDZEICHEN®</sup> ultrafiltration units (Pall Deutschland GmbH Holding, Dreieich, Germany).

Concentrated samples were mixed with a storage buffer containing 20 mM TrisHCl (pH 8.0 at 20° C), 100 mM KCl, 1 mM EDTA, 0.5% (v/v) Nonidet P-40 substitute, 0.5% (v/v) Tween 20 and 50% (v/v) glycerol. Chimeric polymerase preparations were stored at  
10 -20° C. In some cases, the cleared lysate of the polymerase preparation was directly used for subsequent analysis; chimeric polymerase preparations were then stored at +4°C.

#### EXAMPLE 5: 5'-3' Polymerase Activity of Thermostable Chimeric DNA Polymerases

To demonstrate the polymerase activity of thermostable chimeric DNA  
15 polymerases produced from Example 4, an assay for measuring primer extension activity was performed. This assay is based on the difference in mobility of single- versus double-strand DNA molecules on an agarose gel in the presence of a DNA intercalating dye. Annealing of a primer to a single-stranded DNA molecule creates a priming site for a DNA polymerase. The primer is then extended by the polymerase, converting the single-strand  
20 DNA into double-strand molecules. The extension rate is dependent upon the polymerase used. The final amount of DNA extension (i.e., polymerization) is dependent on the amount of polymerase provided, the extension rate of the polymerase, and the length of time the reaction is allowed to proceed.

All polymerization reaction mixtures contained 50 ng M13mp18 DNA (20 fmol;  
25 7250 nt), 0.1 µM 30-mer oligonucleotide primer 5'-TTTCCCAGTCACGACGTTGTAAAACGACGG-3' (SEQ ID NO: 13), and 50 µM of each dNTP in 10 µl of 10 mM Tris HCl.

Polymerization reactions containing *Taq* DNA polymerase and a thermostable chimeric DNA polymerase were performed in 1x PCR buffer (Qiagen, Valencia, CA).

30 *Taq* DNA polymerase was used for external standard reactions (0.05, 0.03, 0.01 units) in order to determine polymerase activity of the thermostable chimeric DNA polymerases. DNA polymerases were diluted in the reaction buffer containing 1 µg/ml bovine serum albumin (BSA) to compensate for possible protein interactions with the surface of the polypropylene tube.

35 The assay was performed in a MJ Research PTC-200 Thermocycler (Biozym, Hess. Oldendorf, Germany) or a Biometra Unoll Thermocycler (Biometra, Göttingen,

Germany). The thermal program consisted of a 10 sec. denaturation step 94° C; a 30 sec. annealing step at 55° C; and a 3 min. polymerization step at 72° C. Heating of the reaction mixture to 94° C was done to destroy possible secondary structures of the single-stranded M13 DNA and to facilitate specific primer annealing during the lowering of  
5 reaction temperature to 55° C.

Results of primer extension reactions at 72° C were reproducible. After completing the reaction, reaction products were mixed with 1 µl gel loading solution (50% Glycerol, 1x TAE buffer, 0.02 mg/ml Bromphenol blue) and loaded on a 1% agarose gel containing 0.5 µg/ml ethidium bromide. The gel was run at 80 mA for 15 min in 1x TAE buffer. These  
10 conditions facilitated discrimination between extended- (ds) and non-extended (ss) M13 DNA fragments. The results, as represented in Fig 1, illustrate the polymerase activity of the thermostable chimeric polymerase is comparable to that of wild type *Taq* polymerase.

#### EXAMPLE 6: Thermostability of Chimeric DNA Polymerases

15 The primer extension assay described in Example 5 was also used to measure the resilience of chimeric DNA polymerases to thermal degradation (i.e., thermostability). Heat-treatment of chimeric DNA polymerases (0.2 units) consisted of incubation of the enzyme for 0, 10, 15, 30, 60, 90 and 120 min at 90°C, followed by primer extension at 72°C. Polymerase activity of heat-treated chimeric polymerase was compared to  
20 untreated chimeric DNA polymerase based on the amount of polymerized (i.e., double strand) M13 DNA. The same assay was performed, under identical reaction conditions, on identical amounts of *Taq* DNA polymerase, as a standard. A control consisted of a polymerase reaction mixture, without any DNA polymerase. After completing the reaction, reaction products were mixed with 1 µl gel loading solution (50% Glycerol, 1x TAE buffer,  
25 0.02 mg/ml Bromphenol blue), and loaded on a 1% agarose gel containing 0.5 µg/ml ethidium bromide. The gel was run at 80 mA for 15 min in 1x TAE buffer. The results, presented in Fig 2 and quantified in Table 2 below, are representative of the thermostability assay.

**Table 2. Thermostability of chimeric polymerase compared to *Taq* polymerase**

Incubation at 90°C (min.)	<i>Pho/Taq</i> Chimeric Polymerase % Activity	<i>Taq</i> Polymerase % Activity
0	100	100
10	84	99
15	84	89
30	82	74
60	66	69
90	53	31*
120	45	43

\* single non-reproducible data; value expected to be higher

Figure 2 and Table 2 confirm the thermostability of the chimeric polymerase of the present invention. Table 2 illustrates that although the activity of the chimeric DNA polymerase shows an initial drop in activity (within the first 10 min at 90°C) greater than that of *Taq* DNA polymerase, the overall thermostability is comparable to *Taq* DNA polymerase. Chimeric DNA polymerase of the invention displays the same half life at 90°C as *Taq* DNA polymerase (approximately 90 min).

The thermostability assay was also performed under extreme temperature conditions. The primer extension assay was run after heat-treatment at 95°C for 0, 3, 5, and 10 min. The results, quantified in Table 3 below, are representative of the 95°C thermostability assay, and further confirm that the chimeric DNA polymerase of the present invention is highly thermostable.

**Table 3. Thermostability of chimeric polymerase**

Incubation at 95°C (min.)	<i>Pho/Taq</i> Chimeric Polymerase % Activity
0	100
3	100
5	86
10	86

These results confirm the thermostability of the chimeric DNA polymerase of the present invention, making it useful for *in vitro* reactions under heat denaturing conditions such as PCR, without requiring successive addition of enzyme at each cycle of the PCR program.

**EXAMPLE 7: 3'-5' Exonuclease Activity of Thermostable Chimeric DNA Polymerases**

Fidelity of DNA replication is based on a two step process: misinsertion and misextension. In PCR, if the DNA polymerase inserts an incorrect nucleotide, and the

resulting 3'-mismatched terminus of the growing DNA chain is not extended, the truncated primer extension product cannot be amplified during subsequent PCR cycles since the downstream primer binding site is missing. Additionally, mismatched termini are less efficiently extended than DNA ends harboring the complementary base. DNA

5 polymerases possessing an enzymatically active 3'-5' exonuclease domain are capable of removing a misincorporated nucleotide, thus increasing fidelity of the PCR product and increasing primer extension efficiency.

A PCR and restriction endonuclease digestion assay, developed to assess the ability of thermostable DNA polymerases to remove mismatched primer termini by 3'-5' exonuclease activity, was performed using the protocol disclosed in U.S. Patent No. 10 5,491,086 (incorporated by reference). Wild type primers, perfectly matching the *Bam*HI restriction enzyme recognition sequence in the *Taq* polymerase gene, and mutant primers, possessing a 3'-mismatch (employing every possible combination) to the first nucleotide of the *Bam*HI restriction enzyme recognition sequence, were used in side-by-

15 side PCR trials.

Wild type primers to 5'-GCACCCCGCTTGGGCAGAG-3' (SEQ ID NO:14) and 5'-TCCCGCCCCTCCTGGAAGAC-3' (SEQ ID NO:15) yield a 151 bp PCR product that becomes digested upon incubation with *Bam*HI restriction enzyme, generating a 132 bp and 19 bp fragment.

20 Three forward primers containing a single 3'-mismatched nucleotide representing a C:A, C:T, and C:C mismatch to SEQ ID NO:14 were used as mutant primers. Any extension product from these mutant primers would corrupt the *Bam*HI restriction site, rendering the resulting PCR products unaffected by *Bam*HI digestion, thus leaving the 151 bp PCR product intact. The presence of an enzymatically active 3'-5' exonuclease

25 domain, would correct the 3'-mismatched nucleotide of the mutant primer, however, thus restoring the *Bam*HI restriction site, rendering the PCR product susceptible to *Bam*HI digestion, thus producing the 132 bp and 19 bp digestion fragments.

Using this PCR fidelity assay, the chimeric thermostable DNA polymerase was tested for the ability to correct a 3'-primer mismatch during PCR. Chimeric polymerase trials were run in parallel with wild type *Taq* DNA polymerase and *Pfu* DNA polymerase I. 30 The *Taq* DNA polymerase trials served as a negative control, representing a DNA polymerase possessing an enzymatically inactive 3'-5' exonuclease domain (i.e., proofreading capability). The *Pfu* DNA polymerase I trials served as a positive control, representing a thermostable DNA polymerase possessing an enzymatically active 3'-5'

35 exonuclease domain.

PCR mixtures comprised 20 ng plasmid pQE-31 containing the (target) *Taq* polymerase gene sequence; 0.5 units of the test DNA polymerase; 0.4  $\mu$ M of the

appropriate trial primers (wild type vs. mutant primers); 200  $\mu$ M of each dNTP; 1x Qiagen PCR buffer (Qiagen, Valencia, CA) or 1x Pfu reaction buffer (Stratagene, La Jolla, CA) and 1.5 mM  $MgCl_2$  in a final reaction volume of 50  $\mu$ l.

PCR was performed using a MJ Research PTC-200 Thermocycler (Biozym, Hess. Oldendorf, Germany) or a Biometra Unoll Thermocycler (Biometra, Göttingen, Germany). The PCR program consisted of an initial 1 min template denaturation step at 94° C followed by 40 cycles of a 30 sec. denaturation step 94° C; a 30 sec. annealing step at 62° C; and a 1 min. polymerization step at 72° C for 1 min. The PCR concluded with a final prolonged extension step for 2 min. at 72° C.

PCR products were analyzed on a 2% agarose gel by gel electrophoresis (approximately 35 min. at 85 volts) in 1x TAE electrophoresis buffer and Ethidium bromide. PCR products were visualized using UV irradiation, and quantified using the 200 bp DNA fragment of the Low DNA MassSONDZEICHEN™ Ladder (Life Technologies, Gaithersburg, MD, USA) as standard by gel densitometry. PCR products were purified using QIAquick™ PCR Purification Kit (Qiagen, Valencia, CA).

Identical amounts of PCR product were digested in the same final reaction volume using 1 unit *Bam*HI (Life Technologies, Gaithersburg, MD, USA) per 100 ng PCR product and corresponding reaction buffer. Restriction digest was performed for 90 min. at 37° C. Digestion products were analyzed on a 4% Metaphor<sup>SONDZEICHEN®</sup> agarose gel (Biozym, Hess. Oldendorf, Germany). Fig. 3. is representative of the results of the 3'-5' exonuclease activity assay.

Fig 3(A) illustrates the PCR product of the three nucleic acid polymerases (*Taq* polymerase, *Pfu* polymerase, and the thermostable chimeric polymerase) using wild type primers. Alternating lanes represent undigested PCR product and PCR product subjected to *Bam*HI digestion. Undigested product shows the intact 151 bp PCR product. Digestion treated product shows the 132 bp digestion fragment.

Fig 3(B) illustrates the PCR product of the three polymerases (*Taq* polymerase, *Pfu* polymerase, and the thermostable chimeric polymerase) using mutant primers. Once again, alternating lanes represent undigested PCR product and PCR product subjected to *Bam*HI digestion. *Taq* polymerase PCR product was unaffected by *Bam*HI digestion (lanes 3 and 5), due to the lack of a *Bam*HI site resulting for normal extension of the mutant primer. *Pfu* polymerase PCR product was effectively digested by *Bam*HI (lanes 7 and 9), producing the expected 132 bp digestion fragment. These results are indicative of the proofreading ability (i.e., 3'-5' exonuclease activity) of *Pfu* polymerase, which corrected the nucleotide mismatch of the mutant primer, thus restoring the *Bam*HI site of the template DNA.

The thermostable chimeric polymerase PCR product displayed results similar to the *Pfu* polymerase PCR product. The chimeric polymerase PCR product was also effectively digested by *Bam*HI (lanes 11 and 13), producing the expected 132 bp digestion fragment and indicative of polymerase proofreading ability. These results confirm that the thermostable chimeric polymerase, which possesses the 5'-3' polymerase domain of *Taq* polymerase, also possesses an enzymatically active 3'-5' exonuclease domain not naturally occurring in *Taq* polymerase.

**EXAMPLE 8: PCR efficiency of Thermostable Chimeric DNA Polymerases**

PCR efficiency of a DNA polymerase can be described as the combined effect of primer extension activity and processivity of the enzyme. PCR efficiency of the thermostable chimeric DNA polymerase was tested in comparison with *Taq* DNA polymerase, known to possess a higher PCR efficiency than common proofreading polymerases, and *Pfu* DNA polymerase (both serving as controls).

One unit of the respective polymerase was used to amplify a 750 bp large product from human genomic DNA using a thermocycling profile with varying primer extension times at 72°C. Limiting primer extension time was used to measure polymerase efficiency in PCR, using the same amount of enzyme activity in the assay. *Taq* DNA polymerase was assayed in its optimized PCR buffer (Qiagen, Valencia, CA), a *Pho/Taq* thermostable chimeric DNA polymerase was used in a 1x buffer consisting of 50 mM TrisHCl (pH 8.9 at room temperature), 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and *Pfu* DNA polymerase was used in the reaction buffer supplied with the enzyme (Stratagene, La Jolla, CA). All reactions contained 1 unit of enzyme, 0.4 µM of each primer, 200 µM of each dNTP, and a final MgCl<sub>2</sub> concentration of 1.5 mM (*Taq* polymerase, chimeric DNA polymerase) or 2.0 mM (*Pfu* polymerase).

Thermocycling was performed in a Biometra Uno thermocycler using the following cycling conditions: initial denaturation at 94°C for 3 min followed by a denaturation step at 94°C for 30 sec, an annealing step at 60°C for 30 sec, and a primer extension step at 72°C for 1 min, 30 sec, 10 sec or 5 sec. The reaction proceeded for 34 cycles, and concluded with a final extension step at 72°C for 10 min.

The results are depicted in Figure 4. *Taq* DNA polymerase (A) shows a high PCR efficiency even when primer extension time is as low as 5 sec. The thermostable chimeric DNA polymerase (B) shows a higher PCR efficiency than *Taq* polymerase at extension times of 1 min and 30 sec, but a slightly lower efficiency than *Taq* polymerase at 5 sec extension time. *Pfu* DNA polymerase I (C) generates a visible PCR product only when using the 1 min extension time.

These results indicate that the overall processivity of the chimeric polymerase is comparable to that of *Taq* DNA polymerase, and is dramatically better than *Pfu* DNA polymerase I. The thermostable chimeric polymerase of the present invention performs as well as *Taq* DNA polymerase (the standard enzyme of PCR protocols) , and  
5 outperforms *Pfu* DNA polymerase I (the standard enzyme for high fidelity PCR protocols). In addition, the thermostable chimeric polymerase of the present invention combines the beneficial features of each of the standard enzymes for PCR protocols formerly not obtained with either *Taq* DNA polymerase or proofreading polymerases: removal of misincorporated nucleotides required for high fidelity PCR, and high PCR efficiency.

10

#### References

- Barnes, PNAS USA 91:2216-2220 (1994).  
Barnes. U.S. Pat. No. 5,436,149 (1995).  
15 Bedford et al., WO 97/29209 (14 August 1997).  
Bernard et al., Cell 59:219 (1989).  
Eom et al., Nature 382: 278-281 (1996)  
Flaman et al. N.A.R. 22:3259-3260 (1994).  
Frey et al., WO 99/47649 (23 September 1999).  
20 Gelfand et al. U.S. Pat. No. 4,889,818 (1989).  
Gelfand et al. U.S. Pat. No. 5,079,352 (1992).  
Gelfand et al. U.S. Pat. No. 5,491,086 (1996).  
Gelfand et al., EP 0 892 058 A2 (20 January 1999).  
Gyllensten et al. U.S. Pat. No. 5,066,584 (1991).  
25 Ho et al. U.S. Pat. No. 5,023,171 (1991).  
Innes et al. U.S. Pat. No. 5,075,216 (1991).  
Innes. U.S. Pat. No. 5,091,310 (1992).  
Jacobson et al., Eur. J. Biochem. 45:623 (1974).  
Joyce and Grindley, PNAS USA 80:1830 (1983).  
30 Joyce and Steitz, Annu. Rev. Biochem. 63:777-822 (1994).  
Kiefer et al., Structure 5: 95-108 (1997).  
Klenow and Henningsen, PNAS USA 65:168 (1970).  
Longley et al. N.A.R. 18:7317-7322 (1990).  
Lawyer et al., J. Biol. Chem. 264:6427-6437 (1989).  
35 Mendelman et al., J. Biol. Chem. 265(4):2338-2346 (1990).  
Mullis et al. U.S. Pat. No. 4,683,195 (1987).  
Mullis, U.S. Pat. No. 4,683,202 (1987).

Mullis et al. U.S. Pat. No. 4,800,159 (1989).

Mullis et al. U.S. Pat. No. 4,965,188 (1990).

Park et al., Mol. Cells 7(3):419-424 (1997).

Petruska et al., PNAS USA 85:6252-6256 (1988).

5 Silver et al. U.S. Pat. No. 5,104,792 (1992).

Each of the publications mentioned herein is incorporated by reference.

10

15

20

25

30

35

40



We claim:

1. A chimeric nucleic acid polymerase comprising at least two enzymatically active domains, wherein at least two of said domains are non-naturally associated, and said chimeric nucleic acid polymerase is thermostable.
2. The chimeric nucleic acid polymerase of claim 1, wherein said domains are derived from enzymes of different species.
3. The polymerase of claim 1, wherein said domains are selected from the group consisting of: 5'-3' exonuclease domain, 3'-5' exonuclease domain, and 5'-3' polymerase domain.
4. The polymerase of claim 3, wherein at least one of said domains is a 5'-3' polymerase domain.
5. The polymerase of claim 3, wherein at least one of said domains is a 3'-5' exonuclease domain.
6. The polymerase of claim 1, wherein at least one of said enzymatically active domains is isolated from a DNA polymerase produced by a thermophilic organism.
7. The polymerase of claim 1, wherein at least one of said enzymatically active domains is isolated from a DNA polymerase produced by an organism of a genus selected from the group of genera consisting of: *Thermus*, *Thermococcus*, *Thermotoga*, *Pyrococcus*, *Pyrodictium*, *Bacillus*, *Sulfolobus*, and *Methanobacterium*.
8. The polymerase of claim 1, wherein at least one of said enzymatically active domains is isolated from a polymerase selected from the group of DNA polymerases consisting of:  
*Tac* polymerase; *Taq* polymerase; *Tba* polymerase; *Tbr* polymerase; *Tfi* polymerase;  
*Tfl* polymerase; *Tfu* polymerase; *Tih* polymerase; *Tli* polymerase; *Tpac* polymerase;  
*Tru* polymerase; *Tth* polymerase; *Pab* polymerase; *Pfu* polymerase; *Phe* polymerase;  
*Pho* polymerase; *Pko* polymerase; *Poc* polymerase; *Psp* polymerase; *Pwo* polymerase;  
*Tma* polymerase; *Tne* polymerase; *Bst* polymerase; *Sac* polymerase; *Sso* polymerase;

*Mth* polymerase; *Kod* polymerase; *ES4* polymerase;  
and mutants, variants, and derivatives thereof.

9. The polymerase of claim 1, wherein said polymerase comprises a 3'-5' exonuclease domain and a 5'-3' polymerase domain.
10. The polymerase of claim 9 wherein said 5'-3' polymerase domain is selected from the group consisting of; a 5'-3' polymerase domain of *Taq* DNA polymerase; a 5'-3' polymerase domain of *Tth* DNA polymerase, and mutants, variants, or derivatives thereof.
11. The polymerase of claim 9 wherein said 5'-3' polymerase domain comprises a Stoffel fragment of *Taq* DNA polymerase or a mutant, variant, or derivative thereof.
12. The polymerase of claim 10 wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1).
13. The polymerase of claim 10 wherein said 5'-3' polymerase domain comprises amino acid residues 271 to 832 of *Taq* DNA polymerase (SEQ ID NO:7).
14. The polymerase of claim 10 wherein said 5'-3' polymerase domain comprises amino acid residues 273 to 834 of *Tth* DNA polymerase (SEQ ID NO:2).
15. The polymerase of claim 9 wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pho* DNA polymerase or a mutant, variant, or derivative thereof.
16. The polymerase of claim 15 wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pho* DNA polymerase (SEQ ID NO:3).
17. The polymerase of claim 9 wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pwo* DNA polymerase or a mutant, variant, or derivative thereof.
18. The polymerase of claim 17 wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pwo* DNA polymerase (SEQ ID NO:4).
19. The polymerase of claim 17 wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 421 of *Pwo* DNA polymerase (SEQ ID NO:5).

20. The polymerase of claim 9 wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Sso* DNA polymerase, mutants, variants, or derivatives thereof.
21. The polymerase of claim 20 wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 508 of *Sso* DNA polymerase (SEQ ID NO:6).
22. The polymerase of claim 9 wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Tpac* DNA polymerase, mutants, variants, or derivatives thereof.
23. The polymerase of claim 22 wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 395 of *Tpac* DNA polymerase (SEQ ID NO:16).
24. The polymerase of claim 9 wherein said 5'-3' polymerase domain comprises a 5'-3' polymerase domain of *Taq* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pho* DNA polymerase or mutant, variant, or derivative thereof.
25. The polymerase of claim 24 wherein, said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pho* DNA polymerase (SEQ ID NO:3).
26. The polymerase of claim 9 wherein said 5'-3' polymerase domain comprises a 5'-3' polymerase domain of *Taq* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pwo* DNA polymerase or mutant, variant, or derivative thereof.
27. The polymerase of claim 26 wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pwo* DNA polymerase (SEQ ID NO:4).
28. The polymerase of claim 26 wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 421 of *Pwo* DNA polymerase (SEQ ID NO:5).

29. The polymerase of claim 9 wherein said 5'-3' polymerase domain comprises a DNA polymerase domain of *Taq* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Sso* DNA polymerase or mutant, variant, or derivative thereof.
30. The polymerase of claim 29 wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 508 of *Sso* DNA polymerase (SEQ ID NO:6).
31. The polymerase of claim 9 wherein said 5'-3' polymerase domain comprises a DNA polymerase domain of *Taq* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Tpac* DNA polymerase or mutant, variant, or derivative thereof.
32. The polymerase of claim 31 wherein said 5'-3' polymerase domain comprises amino acid residues 271 to 832 of *Taq* DNA polymerase (SEQ ID NO:7), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 395 of *Tpac* DNA polymerase (SEQ ID NO:16).
33. The polymerase of claim 9 wherein said 5'-3' polymerase domain comprises a DNA polymerase domain of *Tth* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pho* DNA polymerase or mutant, variant, or derivative thereof.
34. The polymerase of claim 33 wherein said 5'-3' polymerase domain comprises amino acid residues 273 to 834 of *Tth* DNA polymerase (SEQ ID NO:2), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pho* DNA polymerase (SEQ ID NO:3).
35. The polymerase of claim 9 comprising a polypeptide selected from the group consisting of: SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:17; and mutants, variants, or derivatives thereof.
36. An isolated polynucleotide encoding a thermostable chimeric nucleic acid polymerase comprising at least two enzymatically active domains, wherein at least two of said domains are non-naturally associated.
37. The isolated polynucleotide of claim 36, wherein said domains are derived from different species.

38. The isolated polynucleotide of claim 36, wherein said domains are selected from the group consisting of: 5'-3' exonuclease domain, 3'-5' exonuclease domain, and 5'-3' polymerase domain.
39. The isolated polynucleotide of claim 36, wherein at least one of said enzymatically active domains is isolated from a DNA polymerase produced by a thermophilic organism.
40. The isolated polynucleotide of claim 36, wherein at least one of said enzymatically active domains is isolated from a DNA polymerase produced by an organism of a genus selected from the group of genera consisting of: *Thermus*, *Thermococcus*, *Thermotoga*, *Pyrococcus*, *Pyrodictium*, *Bacillus*, *Sulfolobus*, and *Methanobacterium*.
41. The isolated polynucleotide of claim 36, wherein at least one of said enzymatically active domains is isolated from a polymerase selected from the group of DNA polymerases consisting of:  
*Tac* polymerase; *Taq* polymerase; *Tba* polymerase; *Tbr* polymerase; *Tfi* polymerase;  
*Tfl* polymerase; *Tfu* polymerase; *Tih* polymerase; *Tli* polymerase; *Tpac* polymerase;  
*Tru* polymerase; *Tth* polymerase; *Pab* polymerase; *Pfu* polymerase; *Phe* polymerase;  
*Pho* polymerase; *Pko* polymerase; *Poc* polymerase; *Psp* polymerase; *Pwo* polymerase;  
*Tma* polymerase; *Tne* polymerase; *Bst* polymerase; *Sac* polymerase; *Sso* polymerase;  
*Mth* polymerase; *Kod* polymerase; *ES4* polymerase;  
and mutants, variants, and derivatives thereof.
42. The isolated polynucleotide of claim 36, wherein said polymerase comprises a 3'-5' exonuclease domain and a 5'-3' polymerase domain.
43. The isolated polynucleotide of claim 42, wherein said 5'-3' polymerase domain is selected from the group consisting of; a 5'-3' polymerase domain of *Taq* DNA polymerase; a 5'-3' polymerase domain of *Tth* DNA polymerase, and mutants, variants, or derivatives thereof.

44. The isolated polynucleotide of claim 42, wherein said 5'-3' polymerase domain comprises a Stoffel fragment of *Taq* DNA polymerase or a mutant, variant, or derivative thereof.
45. The isolated polynucleotide of claim 43, wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1).
46. The isolated polynucleotide of claim 43, wherein said 5'-3' polymerase domain comprises amino acid residues 271 to 832 of *Taq* DNA polymerase (SEQ ID NO:7).
47. The isolated polynucleotide of claim 43, wherein said 5'-3' polymerase domain comprises amino acid residues 273 to 834 of *Tth* DNA polymerase (SEQ ID NO:2).
48. The isolated polynucleotide of claim 42, wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pho* DNA polymerase or a mutant, variant, or derivative thereof.
49. The isolated polynucleotide of claim 48, wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pho* DNA polymerase (SEQ ID NO:3).
50. The isolated polynucleotide of claim 42, wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pwo* DNA polymerase or a mutant, variant, or derivative thereof.
51. The isolated polynucleotide of claim 50, wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pwo* DNA polymerase (SEQ ID NO:4).
52. The isolated polynucleotide of claim 50, wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 421 of *Pwo* DNA polymerase (SEQ ID NO:5).
53. The isolated polynucleotide of claim 42, wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Sso* DNA polymerase, mutants, variants, or derivatives thereof.
54. The isolated polynucleotide of claim 53, wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 508 of *Sso* DNA polymerase (SEQ ID NO:6).
55. The isolated polynucleotide of claim 42, wherein said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Tpac* DNA polymerase, mutants, variants, or derivatives thereof.

56. The isolated polynucleotide of claim 55, wherein said 3'-5' exonuclease domain comprises amino acid residues 1 to 395 of *Tpac* DNA polymerase (SEQ ID NO:16).
57. The isolated polynucleotide of claim 42, wherein said 5'-3' polymerase domain comprises a 5'-3' polymerase domain of *Taq* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pho* DNA polymerase or mutant, variant, or derivative thereof.
58. The isolated polynucleotide of claim 57, wherein, said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pho* DNA polymerase (SEQ ID NO:3).
59. The isolated polynucleotide of claim 42, wherein said 5'-3' polymerase domain comprises a 5'-3' polymerase domain of *Taq* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pwo* DNA polymerase or mutant, variant, or derivative thereof.
60. The isolated polynucleotide of claim 59, wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pwo* DNA polymerase (SEQ ID NO:4).
61. The isolated polynucleotide of claim 59, wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 421 of *Pwo* DNA polymerase (SEQ ID NO:5).
62. The isolated polynucleotide of claim 42, wherein said 5'-3' polymerase domain comprises a DNA polymerase domain of *Taq* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Sso* DNA polymerase or mutant, variant, or derivative thereof.
63. The isolated polynucleotide of claim 62, wherein said 5'-3' polymerase domain comprises amino acid residues 281 to 832 of *Taq* DNA polymerase (SEQ ID NO:1), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 508 of *Sso* DNA polymerase (SEQ ID NO:6).
64. The isolated polynucleotide of claim 42, wherein said 5'-3' polymerase domain comprises a DNA polymerase domain of *Taq* DNA polymerase or mutant, variant, or

derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Tpac* DNA polymerase or mutant, variant, or derivative thereof.

65. The isolated polynucleotide of claim 64, wherein said 5'-3' polymerase domain comprises amino acid residues 271 to 832 of *Taq* DNA polymerase (SEQ ID NO:7), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 395 of *Tpac* DNA polymerase (SEQ ID NO:16).
66. The isolated polynucleotide of claim 42, wherein said 5'-3' polymerase domain comprises a DNA polymerase domain of *Tth* DNA polymerase or mutant, variant, or derivative thereof, and said 3'-5' exonuclease domain comprises a 3'-5' exonuclease domain of *Pho* DNA polymerase or mutant, variant, or derivative thereof.
67. The isolated polynucleotide of claim 66, wherein said 5'-3' polymerase domain comprises amino acid residues 273 to 834 of *Tth* DNA polymerase (SEQ ID NO:2), and said 3'-5' exonuclease domain comprises amino acid residues 1 to 396 of *Pho* DNA polymerase (SEQ ID NO:3).
68. The isolated polynucleotide of claim 42, encoding a polypeptide selected from the group consisting of: SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:17; and mutants, variants, or derivatives thereof.
69. A vector comprising a polynucleotide wherein said polynucleotide is selected from the group of polynucleotides of claims 36 to 68.
70. The vector of claim 69 wherein said vector is an expression vector.
71. A recombinant host cell transformed with a vector, said vector comprising a polynucleotide selected from the group of polynucleotides of claims 36 to 68.
72. A kit for polymerization of nucleic acid comprising; a chimeric nucleic acid polymerase according to any one of claims 1 to 35.
73. The kit of claim 72 further comprising at least one reagent suitable for nucleic acid polymerization.
74. The kit of claim 73 wherein said nucleic acid polymerization is nucleic acid amplification.
75. The kit of claim 73 wherein said reagent is selected from the group consisting of; one or more additional enzymes, one or more oligonucleotide primers, a nucleic acid



template, any one or more nucleotide bases, an appropriate buffering agent, and a salt.

76. The kit of claim 75 wherein said reagent is phosphatase.

77. A process for nucleic acid polymerization, comprising the steps of:

- (a) providing a chimeric nucleic acid polymerase according to any one of claims 1 to 35; and
- (b) contacting said chimeric polymerase with a nucleic acid template under conditions sufficient to allow polymerization of a nascent nucleic acid complementary to said nucleic acid template.

78. A process for synthesizing a recombinant nucleic acid encoding a thermostable chimeric nucleic acid polymerase having at least two enzymatically active domains, wherein at least two of said domains are non-naturally associated, comprising the steps of:

- (a) isolating nucleic acid encoding a first enzymatically active domain;
- (b) isolating nucleic acid encoding a second enzymatically active domain, wherein said second domain is not naturally associated with said first domain; and
- (c) genetically combining said nucleic acid encoding a first enzymatically active domain with said nucleic acid encoding a second enzymatically active domain to form a chimeric nucleic acid encoding a thermostable nucleic acid polymerase, wherein said first domain and said second domain are enzymatically active.

79. The process of claim 78, wherein, at least one of said domains is a 5'-3' polymerase domain.

80. The process of claim 78, wherein, at least one of said domains is a 3'-5' exonuclease domain.

81. A process for producing a thermostable chimeric nucleic acid polymerase having at least two enzymatically active domains, wherein at least two of said domains are non-naturally associated, comprising the steps of:

- (a) culturing a host cell containing nucleic acid encoding said chimeric polymerase under conditions suitable for expression of said chimeric polymerase; and isolating said chimeric polymerase expressed from said cell culture.

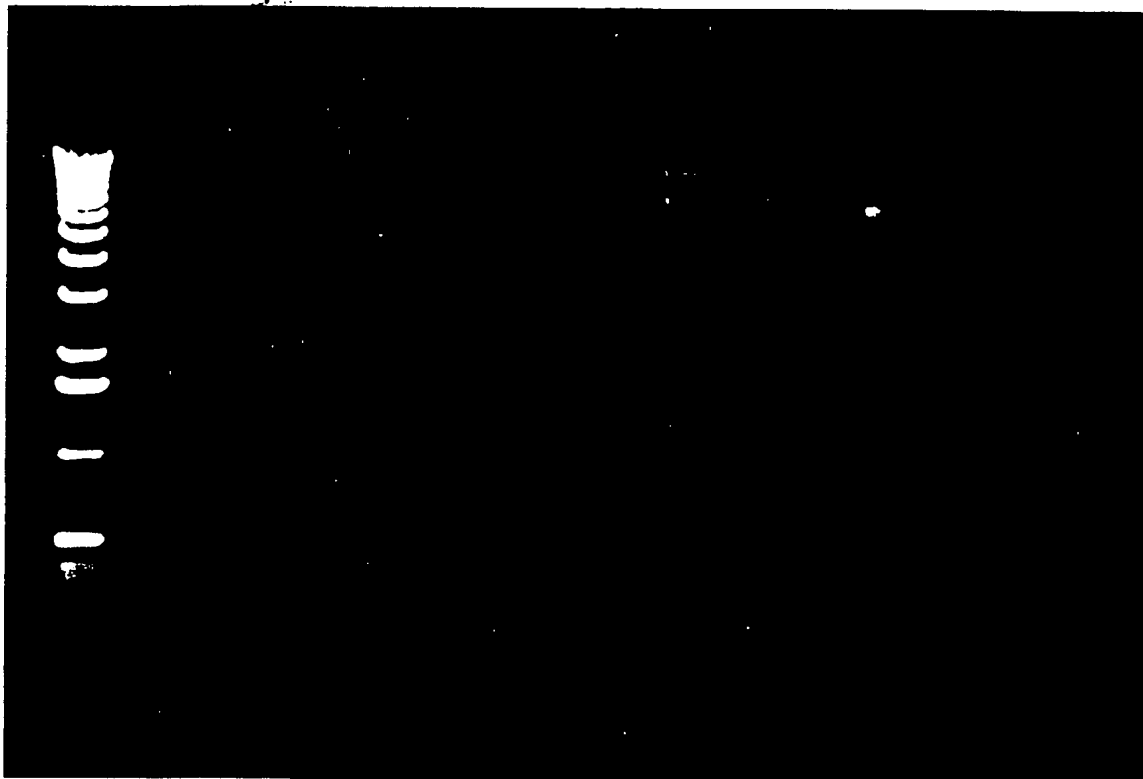


FIGURE 1

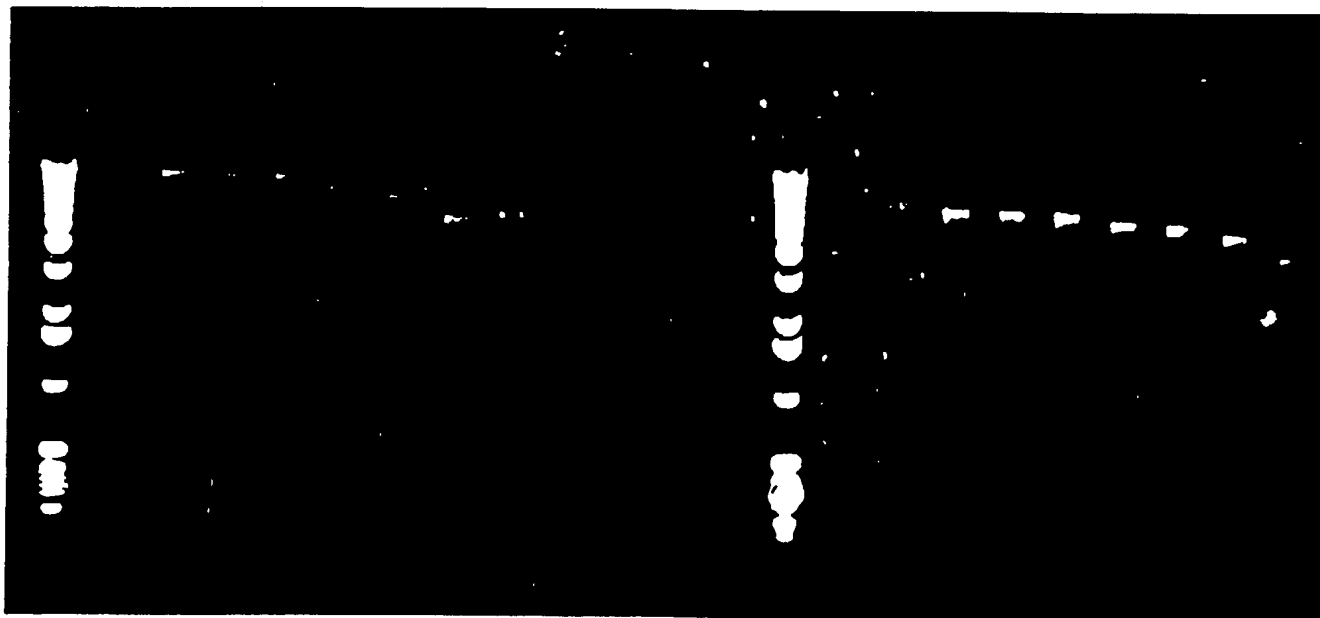
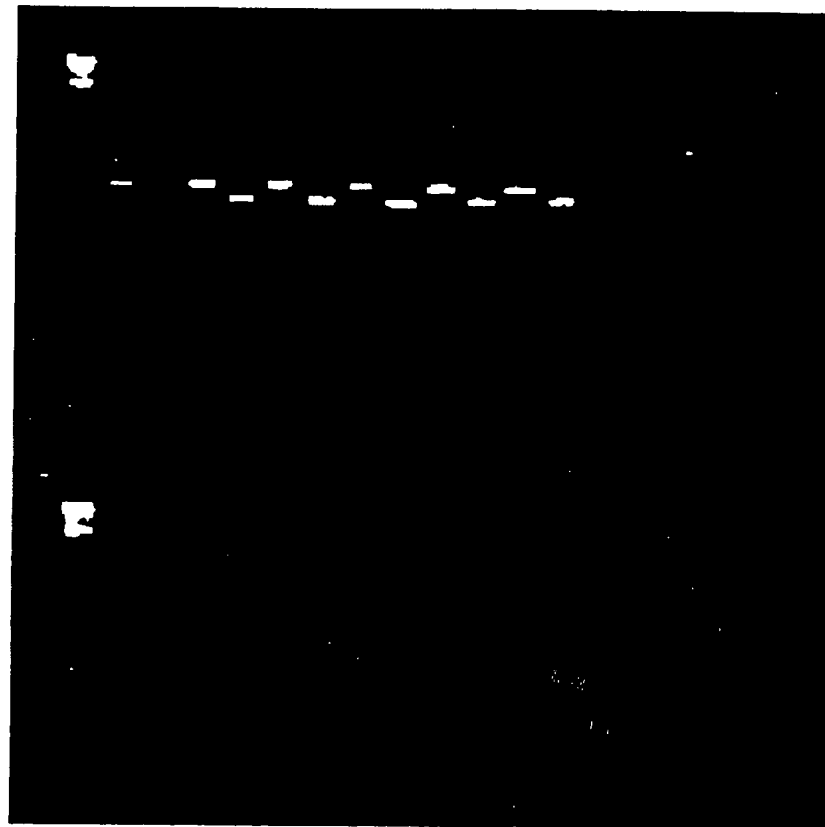


FIGURE 2

(A)  
Wild Type Primer  
Extension

(B)  
Mutant Primer  
Extension



┌───┐ ┌───┐ ┌───┐  
Taq pol. Pfu pol. Chimeric pol.

FIGURE 3

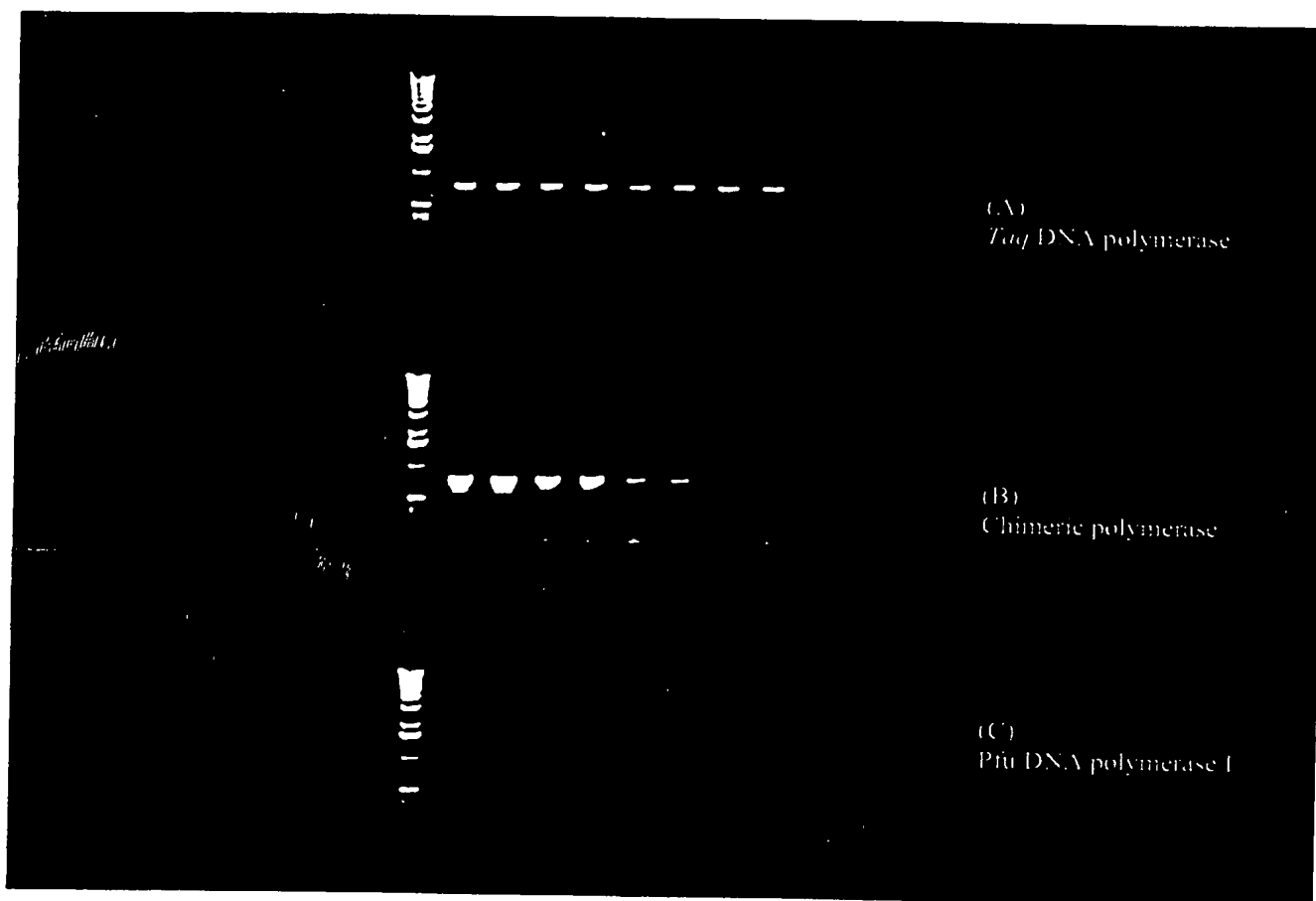


FIGURE 4

## SEQUENCE LISTING

<110> Qiagen GmbH  
 5 Loffert, Dirk  
 Missel, Andreas  
 Kang, Jie  
  
 <120> Thermostable Chimeric Nucleic Acid Polymerases and Uses  
 10 Thereof  
  
 <130> PA058-PCT  
  
 <140> (not yet assigned)  
 15 <141> (concurrently herewith)  
  
 <150> US 09/506,153  
 <151> 2000-02-17  
  
 20 <160> 17  
  
 <170> Microsoft Word 97 SR-1  
  
 <210> 1  
 25 <211> 552  
 <212> PRT  
 <213> Thermus aquaticus  
  
 <400> 1  
 30 Leu Leu His Glu Phe Gly Leu Leu Glu Ser Pro Lys Ala Leu Glu Glu  
     1                    5                    10                    15  
  
 Ala Pro Trp Pro Pro Pro Glu Gly Ala Phe Val Gly Phe Val Leu Ser  
                     20                    25                    30  
 35 Arg Lys Glu Pro Met Trp Ala Asp Leu Leu Ala Leu Ala Ala Arg  
             35                    40                    45  
  
 Gly Gly Arg Val His Arg Ala Pro Glu Pro Tyr Lys Ala Leu Arg Asp  
 40            50                    55                    60  
  
 Leu Lys Glu Ala Arg Gly Leu Leu Ala Lys Asp Leu Ser Val Leu Ala

	65		70		75		80
	Leu Arg Glu Gly Leu Gly Leu Pro Pro Gly Asp Asp Pro Met Leu Leu						
			85		90		95
5	Ala Tyr Leu Leu Asp Pro Ser Asn Thr Thr Pro Glu Gly Val Ala Arg						
			100		105		110
	Arg Tyr Gly Gly Glu Trp Thr Glu Glu Ala Gly Glu Arg Ala Ala Leu						
10			115		120		125
	Ser Glu Arg Leu Phe Ala Asn Leu Trp Gly Arg Leu Glu Gly Glu Glu						
			130		135		140
15	Arg Leu Leu Trp Leu Tyr Arg Glu Val Glu Arg Pro Leu Ser Ala Val						
			145		150		155
							160
	Leu Ala His Met Glu Ala Thr Gly Val Arg Leu Asp Val Ala Tyr Leu						
			165		170		175
20	Arg Ala Leu Ser Leu Glu Val Ala Glu Glu Ile Ala Arg Leu Glu Ala						
			180		185		190
	Glu Val Phe Arg Leu Ala Gly His Pro Phe Asn Leu Asn Ser Arg Asp						
25			195		200		205
	Gln Leu Glu Arg Val Leu Phe Asp Glu Leu Gly Leu Pro Ala Ile Gly						
			210		215		220
30	Lys Thr Glu Lys Thr Gly Lys Arg Ser Thr Ser Ala Ala Val Leu Glu						
			225		230		235
							240
	Ala Leu Arg Glu Ala His Pro Ile Val Glu Lys Ile Leu Gln Tyr Arg						
			245		250		255
35	Glu Leu Thr Lys Leu Lys Ser Thr Tyr Ile Asp Pro Leu Pro Asp Leu						
			260		265		270
	Ile His Pro Arg Thr Gly Arg Leu His Thr Arg Phe Asn Gln Thr Ala						
40			275		280		285
	Thr Ala Thr Gly Arg Leu Ser Ser Ser Asp Pro Asn Leu Gln Asn Ile						

	290		295		300
	Pro Val Arg Thr	Pro Leu Gly Gln Arg	Ile Arg Arg Ala Phe Ile Ala		
	305	310	315		320
5	Glu Glu Gly Trp	Leu Leu Val Ala Leu Asp Tyr Ser Gln Ile Glu Leu			
		325	330		335
	Arg Val Leu Ala His Leu Ser Gly Asp Glu Asn Leu Ile Arg Val Phe				
10		340	345		350
	Gln Glu Gly Arg Asp Ile His Thr Glu Thr Ala Ser Trp Met Phe Gly				
		355	360		365
15	Val Pro Arg Glu Ala Val Asp Pro Leu Met Arg Arg Ala Ala Lys Thr				
		370	375		380
	Ile Asn Phe Gly Val Leu Tyr Gly Met Ser Ala His Arg Leu Ser Gln				
	385	390	395		400
20	Glu Leu Ala Ile Pro Tyr Glu Glu Ala Gln Ala Phe Ile Glu Arg Tyr				
		405	410		415
	Phe Gln Ser Phe Pro Lys Val Arg Ala Trp Ile Glu Lys Thr Leu Glu				
25		420	425		430
	Glu Gly Arg Arg Arg Gly Tyr Val Glu Thr Leu Phe Gly Arg Arg Arg				
		435	440		445
30	Tyr Val Pro Asp Leu Glu Ala Arg Val Lys Ser Val Arg Glu Ala Ala				
		450	455		460
	Glu Arg Met Ala Phe Asn Met Pro Val Gln Gly Thr Ala Ala Asp Leu				
	465	470	475		480
35	Met Lys Leu Ala Met Val Lys Leu Phe Pro Arg Leu Glu Glu Met Gly				
		485	490		495
	Ala Arg Met Leu Leu Gln Val His Asp Glu Leu Val Leu Glu Ala Pro				
40		500	505		510
	Lys Glu Arg Ala Glu Ala Val Ala Arg Leu Ala Lys Glu Val Met Glu				



515                                      520                                      525  
 Gly Val Tyr Pro Leu Ala Val Pro Leu Glu Val Glu Val Gly Ile Gly  
 530                                      535                                      540  
 5  
 Glu Asp Trp Leu Ser Ala Lys Glu  
 545                                      550  
 10 <210> 2  
 <211> 562  
 <212> PRT  
 <213> *Thermus thermophilus*  
 15 <400> 2  
 Ala Phe Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly  
 1                                      5                                      10                                      15  
 Leu Leu Glu Ala Pro Ala Pro Leu Glu Glu Ala Pro Trp Pro Pro Pro  
 20                                      20                                      25                                      30  
 Glu Gly Ala Phe Val Gly Phe Val Leu Ser Arg Pro Glu Pro Met Trp  
 35                                      40                                      45  
 25 Ala Glu Leu Lys Ala Leu Ala Ala Cys Arg Asp Gly Arg Val His Arg  
 50                                      55                                      60  
 Ala Ala Asp Pro Leu Ala Gly Leu Lys Asp Leu Lys Glu Val Arg Gly  
 65                                      70                                      75                                      80  
 30 Leu Leu Ala Lys Asp Leu Ala Val Leu Ala Ser Arg Glu Gly Leu Asp  
 85                                      90                                      95  
 Leu Val Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro  
 35                                      100                                      105                                      110  
 Ser Asn Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp  
 115                                      120                                      125  
 40 Thr Glu Asp Ala Ala His Arg Ala Leu Leu Ser Glu Arg Leu His Arg  
 130                                      135                                      140

	Asn	Leu	Leu	Lys	Arg	Leu	Glu	Gly	Glu	Glu	Lys	Leu	Leu	Trp	Leu	Tyr	145	150	155	160
5	His	Glu	Val	Glu	Lys	Pro	Leu	Ser	Arg	Val	Leu	Ala	His	Met	Glu	Ala	165	170	175	
	Thr	Gly	Val	Arg	Arg	Asp	Val	Ala	Tyr	Leu	Gln	Ala	Leu	Ser	Leu	Glu	180	185	190	
10	Leu	Ala	Glu	Glu	Ile	Arg	Arg	Leu	Glu	Glu	Glu	Val	Phe	Arg	Leu	Ala	195	200	205	
	Gly	His	Pro	Phe	Asn	Leu	Asn	Ser	Arg	Asp	Gln	Leu	Glu	Arg	Val	Leu	210	215	220	
15	Phe	Asp	Glu	Leu	Arg	Leu	Pro	Ala	Leu	Gly	Lys	Thr	Gln	Lys	Thr	Gly	225	230	235	240
	Lys	Arg	Ser	Thr	Ser	Ala	Ala	Val	Leu	Glu	Ala	Leu	Arg	Glu	Ala	His	245	250	255	
20	Pro	Ile	Val	Glu	Lys	Ile	Leu	Gln	His	Arg	Glu	Leu	Thr	Lys	Leu	Lys	260	265	270	
	Asn	Thr	Tyr	Val	Asp	Pro	Leu	Pro	Ser	Leu	Val	His	Pro	Arg	Thr	Gly	275	280	285	
	Arg	Leu	His	Thr	Arg	Phe	Asn	Gln	Thr	Ala	Thr	Ala	Thr	Gly	Arg	Leu	290	295	300	
30	Ser	Ser	Ser	Asp	Pro	Asn	Leu	Gln	Asn	Ile	Pro	Val	Arg	Thr	Pro	Leu	305	310	315	320
	Gly	Gln	Arg	Ile	Arg	Arg	Ala	Phe	Val	Ala	Glu	Ala	Gly	Trp	Ala	Leu	325	330	335	
35	Val	Ala	Leu	Asp	Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Val	Leu	Ala	His	Leu	340	345	350	
	Ser	Gly	Asp	Glu	Asn	Leu	Ile	Arg	Val	Phe	Gln	Glu	Gly	Lys	Asp	Ile	355	360	365	

His Thr Gln Thr Ala Ser Trp Met Phe Gly Val Pro Pro Glu Ala Val  
 370 375 380

5 Asp Pro Leu Met Arg Arg Ala Ala Lys Thr Val Asn Phe Gly Val Leu  
 385 390 395 400

Tyr Gly Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr  
 405 410 415

10 Glu Glu Ala Val Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys  
 420 425 430

Val Arg Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Lys Arg Gly  
 435 440 445

15 Tyr Val Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Asn  
 450 455 460

Ala Arg Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn  
 20 465 470 475 480

Met Pro Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val  
 485 490 495

25 Lys Leu Phe Pro Arg Leu Arg Glu Met Gly Ala Arg Met Leu Leu Gln  
 500 505 510

Val His Asp Glu Leu Leu Leu Glu Ala Pro Gln Ala Arg Ala Glu Glu  
 515 520 525

30 Val Ala Ala Leu Ala Lys Glu Ala Met Glu Lys Ala Tyr Pro Leu Ala  
 530 535 540

Val Pro Leu Glu Val Glu Val Gly Met Gly Glu Asp Trp Leu Ser Ala  
 35 545 550 555 560

Lys Gly

40  
 <210> 3  
 <211> 396

&lt;212&gt; PRT

&lt;213&gt; Pyrococcus horikoshii

&lt;400&gt; 3

5 Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Asp Gly Lys Pro Ile Ile  
     1                    5                    10                    15  
 Arg Ile Phe Lys Lys Glu Asn Gly Glu Phe Lys Val Glu Tyr Asp Arg  
                     20                    25                    30  
 10 Asn Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Ala Ile  
                     35                    40                    45  
 Asp Glu Ile Lys Lys Ile Thr Ala Gln Arg His Gly Lys Val Val Arg  
 15                    50                    55                    60  
 Ile Val Glu Thr Glu Lys Ile Gln Arg Lys Phe Leu Gly Arg Pro Ile  
     65                    70                    75                    80  
 20 Glu Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Ala Ile  
                     85                    90                    95  
 Arg Asp Lys Ile Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr  
                     100                    105                    110  
 25 Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Thr Pro  
                     115                    120                    125  
 Met Glu Gly Asn Glu Lys Leu Thr Phe Leu Ala Val Asp Ile Glu Thr  
 30                    130                    135                    140  
 Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Val Ile Met Ile  
     145                    150                    155                    160  
 35 Ser Tyr Ala Asp Glu Glu Gly Ala Lys Val Ile Thr Trp Lys Lys Ile  
                     165                    170                    175  
 Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys  
                     180                    185                    190  
 40 Arg Leu Ile Arg Val Ile Lys Glu Lys Asp Pro Asp Val Ile Ile Thr  
                     195                    200                    205

Tyr Asn Gly Asp Asn Phe Asp Phe Pro Tyr Leu Leu Lys Arg Ala Glu  
 210 215 220

5 Lys Leu Gly Ile Lys Leu Leu Leu Gly Arg Asp Asn Ser Glu Pro Lys  
 225 230 235 240

Met Gln Lys Met Gly Asp Ser Leu Ala Val Glu Ile Lys Gly Arg Ile  
 245 250 255

10 His Phe Asp Leu Phe Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr  
 260 265 270

Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu  
 15 275 280 285

Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly  
 290 295 300

20 Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr  
 305 310 315 320

Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ala Arg Leu  
 325 330 335

25 Val Gly Gln Pro Val Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu  
 340 345 350

Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala  
 30 355 360 365

Pro Asn Lys Pro Asp Glu Lys Glu Tyr Glu Arg Arg Leu Arg Glu Ser  
 370 375 380

35 Tyr Glu Gly Gly Tyr Val Lys Glu Pro Glu Lys Gly  
 385 390 395

<210> 4

40 <211> 396

<212> PRT

<213> Pyrococcus woesei

<400> 4

Met	Ile	Leu	Asp	Val	Asp	Tyr	Ile	Thr	Glu	Glu	Gly	Lys	Pro	Val	Ile
1				5					10					15	
5															
Arg	Leu	Phe	Lys	Lys	Glu	Asn	Gly	Lys	Phe	Lys	Ile	Glu	His	Asp	Arg
			20					25					30		
Thr	Phe	Arg	Pro	Tyr	Ile	Tyr	Ala	Leu	Leu	Arg	Asp	Asp	Ser	Lys	Ile
10		35					40					45			
Glu	Glu	Val	Lys	Lys	Ile	Thr	Gly	Glu	Arg	His	Gly	Lys	Ile	Val	Arg
	50					55					60				
15	Ile	Val	Asp	Val	Glu	Lys	Val	Glu	Lys	Lys	Phe	Leu	Gly	Lys	Pro
	65					70					75				80
Thr	Val	Trp	Lys	Leu	Tyr	Leu	Glu	His	Pro	Gln	Asp	Val	Pro	Thr	Ile
			85						90					95	
20															
Arg	Glu	Lys	Val	Arg	Glu	His	Pro	Ala	Val	Val	Asp	Ile	Phe	Glu	Tyr
			100					105					110		
Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro
25		115					120					125			
Met	Glu	Gly	Glu	Glu	Glu	Leu	Lys	Ile	Leu	Ala	Phe	Asp	Ile	Glu	Thr
	130					135					140				
30	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Lys	Gly	Pro	Ile	Ile	Met
	145					150					155				160
Ser	Tyr	Ala	Asp	Glu	Asn	Glu	Ala	Lys	Val	Ile	Thr	Trp	Lys	Asn	Ile
			165						170					175	
35															
Asp	Leu	Pro	Tyr	Val	Glu	Val	Val	Ser	Ser	Glu	Arg	Glu	Met	Ile	Lys
			180					185					190		
Arg	Phe	Leu	Arg	Ile	Ile	Arg	Glu	Lys	Asp	Pro	Asp	Ile	Ile	Val	Thr
40		195					200					205			
Tyr	Asn	Gly	Asp	Ser	Phe	Asp	Phe	Pro	Tyr	Leu	Ala	Lys	Arg	Ala	Glu

	210		215		220	
	Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys					
	225		230		235	240
5	Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile					
		245		250		255
	His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro Thr					
10		260		265		270
	Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu					
		275		280		285
15	Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu Asn					
		290		295		300
	Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr					
	305		310		315	320
20	Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg Leu					
		325		330		335
	Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu					
25		340		345		350
	Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val Ala					
		355		360		365
30	Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu Ser					
		370		375		380
	Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly					
	385		390		395	
35						
	<210> 5					
	<211> 421					
	<212> PRT					
40	<213> Pyrococcus woesei					
	<400> 5					

	Met	Ile	Leu	Asp	Val	Asp	Tyr	Ile	Thr	Glu	Glu	Gly	Lys	Pro	Val	Ile	
	1				5					10					15		
5	Arg	Leu	Phe	Lys	Lys	Glu	Asn	Gly	Lys	Phe	Lys	Ile	Glu	His	Asp	Arg	
				20					25					30			
	Thr	Phe	Arg	Pro	Tyr	Ile	Tyr	Ala	Leu	Leu	Arg	Asp	Asp	Ser	Lys	Ile	
				35				40					45				
10	Glu	Glu	Val	Lys	Lys	Ile	Thr	Gly	Glu	Arg	His	Gly	Lys	Ile	Val	Arg	
		50					55					60					
	Ile	Val	Asp	Val	Glu	Lys	Val	Glu	Lys	Lys	Phe	Leu	Gly	Lys	Pro	Ile	
	65					70					75					80	
15	Thr	Val	Trp	Lys	Leu	Tyr	Leu	Glu	His	Pro	Gln	Asp	Val	Pro	Thr	Ile	
					85					90						95	
	Arg	Glu	Lys	Val	Arg	Glu	His	Pro	Ala	Val	Val	Asp	Ile	Phe	Glu	Tyr	
20				100					105					110			
	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro	
				115				120					125				
25	Met	Glu	Gly	Glu	Glu	Glu	Leu	Lys	Ile	Leu	Ala	Phe	Asp	Ile	Glu	Thr	
		130					135						140				
	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Lys	Gly	Pro	Ile	Ile	Met	Ile	
	145					150					155					160	
30	Ser	Tyr	Ala	Asp	Glu	Asn	Glu	Ala	Lys	Val	Ile	Thr	Trp	Lys	Asn	Ile	
					165					170					175		
	Asp	Leu	Pro	Tyr	Val	Glu	Val	Val	Ser	Ser	Glu	Arg	Glu	Met	Ile	Lys	
35				180					185					190			
	Arg	Phe	Leu	Arg	Ile	Ile	Arg	Glu	Lys	Asp	Pro	Asp	Ile	Ile	Val	Thr	
				195				200					205				
40	Tyr	Asn	Gly	Asp	Ser	Phe	Asp	Phe	Pro	Tyr	Leu	Ala	Lys	Arg	Ala	Glu	
		210					215					220					



Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys  
 225 230 235 240  
 Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile  
 5 245 250 255  
 His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro Thr  
 260 265 270  
 10 Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu  
 275 280 285  
 Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu Asn  
 290 295 300  
 15 Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr  
 305 310 315 320  
 Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg Leu  
 20 325 330 335  
 Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu  
 340 345 350  
 25 Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val Ala  
 355 360 365  
 Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu Ser  
 370 375 380  
 30 Tyr Thr Gly Gly Phe Val Lys Glu Pro Glu Lys Gly Leu Trp Glu Asn  
 385 390 395 400  
 Ile Val Tyr Leu Asp Phe Arg Ala Leu Tyr Pro Ser Ile Ile Ile Thr  
 35 405 410 415  
 His Asn Val Ser Pro  
 420  
 40  
 <210> 6  
 <211> 508

&lt;212&gt; PRT

<213> *Sulfolobus solfataricus*

&lt;400&gt; 6

```

5  Met Thr Lys Gln Leu Thr Leu Phe Asp Ile Pro Ser Ser Lys Pro Ala
    1             5             10             15

    Lys Ser Glu Gln Asn Thr Gln Gln Ser Gln Gln Ser Ala Pro Val Glu
                20             25             30
10  Glu Lys Lys Val Val Arg Arg Glu Trp Leu Glu Glu Ala Gln Glu Asn
        35             40             45

    Lys Ile Tyr Phe Leu Leu Gln Val Asp Tyr Asp Gly Lys Lys Gly Lys
15         50             55             60

    Ala Val Cys Lys Leu Phe Asp Lys Glu Thr Gln Lys Ile Tyr Ala Leu
    65             70             75             80

20  Tyr Asp Asn Thr Gly His Lys Pro Tyr Phe Leu Val Asp Leu Glu Pro
        85             90             95

    Asp Lys Val Gly Lys Ile Pro Lys Ile Val Arg Asp Pro Ser Phe Asp
        100             105             110
25  His Ile Glu Thr Val Ser Lys Ile Asp Pro Tyr Thr Trp Asn Lys Phe
        115             120             125

    Lys Leu Thr Lys Ile Val Val Arg Asp Pro His Ala Val Arg Arg Leu
30         130             135             140

    Arg Asn Asp Val Pro Lys Ala Tyr Glu Ala His Ile Lys Tyr Phe Asn
    145             150             155             160

35  Asn Tyr Met Tyr Asp Ile Gly Leu Ile Pro Gly Met Pro Tyr Val Val
        165             170             175

    Lys Asn Gly Lys Leu Glu Ser Val Tyr Leu Ser Leu Asp Glu Lys Asp
        180             185             190
40  Val Glu Glu Ile Lys Lys Ala Phe Ala Asp Ser Asp Glu Met Thr Arg
        195             200             205

```

	Gln	Met	Ala	Val	Asp	Trp	Leu	Pro	Ile	Phe	Glu	Thr	Glu	Ile	Pro	Lys	
	210						215						220				
5	Ile	Lys	Arg	Val	Ala	Ile	Asp	Ile	Glu	Val	Tyr	Thr	Pro	Val	Lys	Gly	
	225						230				235					240	
	Arg	Ile	Pro	Asp	Ser	Gln	Lys	Ala	Glu	Phe	Pro	Ile	Ile	Ser	Ile	Ala	
					245					250					255		
10	Leu	Ala	Gly	Ser	Asp	Gly	Leu	Lys	Lys	Val	Leu	Val	Leu	Asn	Arg	Asn	
				260					265					270			
	Asp	Val	Asn	Glu	Gly	Ser	Val	Lys	Leu	Asp	Gly	Ile	Ser	Val	Glu	Arg	
15			275					280					285				
	Phe	Asn	Thr	Glu	Tyr	Glu	Leu	Leu	Gly	Arg	Phe	Phe	Asp	Ile	Leu	Leu	
	290						295					300					
20	Glu	Tyr	Pro	Ile	Val	Leu	Thr	Phe	Asn	Gly	Asp	Asp	Phe	Asp	Leu	Pro	
	305					310					315					320	
	Tyr	Ile	Tyr	Phe	Arg	Ala	Leu	Lys	Leu	Gly	Tyr	Phe	Pro	Glu	Glu	Ile	
				325						330					335		
25	Pro	Ile	Asp	Val	Ala	Gly	Lys	Asp	Glu	Ala	Lys	Tyr	Leu	Ala	Gly	Leu	
				340					345					350			
	His	Ile	Asp	Leu	Tyr	Lys	Phe	Phe	Phe	Asn	Lys	Ala	Val	Arg	Asn	Tyr	
30			355					360					365				
	Ala	Phe	Glu	Gly	Lys	Tyr	Asn	Glu	Tyr	Asn	Leu	Asp	Ala	Val	Ala	Lys	
	370						375					380					
35	Ala	Leu	Leu	Gly	Thr	Ser	Lys	Val	Lys	Val	Asp	Thr	Leu	Ile	Ser	Phe	
	385					390					395					400	
	Leu	Asp	Val	Glu	Lys	Leu	Ile	Glu	Tyr	Asn	Phe	Arg	Asp	Ala	Glu	Ile	
				405					410					415			
40	Thr	Leu	Gln	Leu	Thr	Thr	Phe	Asn	Asn	Asp	Leu	Thr	Met	Lys	Leu	Ile	
				420					425					430			

Val Leu Phe Ser Arg Ile Ser Arg Leu Gly Ile Glu Glu Leu Thr Arg  
 435 440 445

5 Thr Glu Ile Ser Thr Trp Val Lys Asn Leu Tyr Tyr Trp Glu His Arg  
 450 455 460

Lys Arg Asn Trp Leu Ile Pro Leu Lys Glu Glu Ile Leu Ala Lys Ser  
 465 470 475 480

10 Ser Asn Ile Arg Thr Ser Ala Leu Ile Lys Gly Lys Gly Tyr Lys Gly  
 485 490 495

Ala Val Val Ile Asp Pro Pro Ala Gly Ile Phe Phe  
 15 500 505

<210> 7  
 <211> 562  
 20 <212> PRT  
 <213> Thermus aquaticus

<400> 7  
 Ala Phe Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly  
 25 1 5 10 15

Leu Leu Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro  
 20 25 30

30 Glu Gly Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp  
 35 40 45

Ala Asp Leu Leu Ala Leu Ala Ala Arg Gly Gly Arg Val His Arg  
 50 55 60

35 Ala Pro Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly  
 65 70 75 80

Leu Leu Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly  
 40 85 90 95

Leu Pro Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro

		100		105		110											
	Ser	Asn	Thr	Thr	Pro	Glu	Gly	Val	Ala	Arg	Arg	Tyr	Gly	Gly	Glu	Trp	
			115					120					125				
5	Thr	Glu	Glu	Ala	Gly	Glu	Arg	Ala	Ala	Leu	Ser	Glu	Arg	Leu	Phe	Ala	
		130					135					140					
	Asn	Leu	Trp	Gly	Arg	Leu	Glu	Gly	Glu	Glu	Arg	Leu	Leu	Trp	Leu	Tyr	
10	145					150					155					160	
	Arg	Glu	Val	Glu	Arg	Pro	Leu	Ser	Ala	Val	Leu	Ala	His	Met	Glu	Ala	
						165				170					175		
15	Thr	Gly	Val	Arg	Leu	Asp	Val	Ala	Tyr	Leu	Arg	Ala	Leu	Ser	Leu	Glu	
						180				185				190			
	Val	Ala	Glu	Glu	Ile	Ala	Arg	Leu	Glu	Ala	Glu	Val	Phe	Arg	Leu	Ala	
			195					200					205				
20	Gly	His	Pro	Phe	Asn	Leu	Asn	Ser	Arg	Asp	Gln	Leu	Glu	Arg	Val	Leu	
		210					215					220					
	Phe	Asp	Glu	Leu	Gly	Leu	Pro	Ala	Ile	Gly	Lys	Thr	Glu	Lys	Thr	Gly	
25	225					230					235					240	
	Lys	Arg	Ser	Thr	Ser	Ala	Ala	Val	Leu	Glu	Ala	Leu	Arg	Glu	Ala	His	
						245				250					255		
30	Pro	Ile	Val	Glu	Lys	Ile	Leu	Gln	Tyr	Arg	Glu	Leu	Thr	Lys	Leu	Lys	
						260				265				270			
	Ser	Thr	Tyr	Ile	Asp	Pro	Leu	Pro	Asp	Leu	Ile	His	Pro	Arg	Thr	Gly	
						275				280			285				
35	Arg	Leu	His	Thr	Arg	Phe	Asn	Gln	Thr	Ala	Thr	Ala	Thr	Gly	Arg	Leu	
		290					295					300					
	Ser	Ser	Ser	Asp	Pro	Asn	Leu	Gln	Asn	Ile	Pro	Val	Arg	Thr	Pro	Leu	
40	305					310				315						320	
	Gly	Gln	Arg	Ile	Arg	Arg	Ala	Phe	Ile	Ala	Glu	Glu	Gly	Trp	Leu	Leu	

					325						330					335
	Val	Ala	Leu	Asp	Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Val	Leu	Ala	His	Leu
				340					345					350		
5	Ser	Gly	Asp	Glu	Asn	Leu	Ile	Arg	Val	Phe	Gln	Glu	Gly	Arg	Asp	Ile
			355					360					365			
	His	Thr	Glu	Thr	Ala	Ser	Trp	Met	Phe	Gly	Val	Pro	Arg	Glu	Ala	Val
10		370					375					380				
	Asp	Pro	Leu	Met	Arg	Arg	Ala	Ala	Lys	Thr	Ile	Asn	Phe	Gly	Val	Leu
	385					390					395				400	
15	Tyr	Gly	Met	Ser	Ala	His	Arg	Leu	Ser	Gln	Glu	Leu	Ala	Ile	Pro	Tyr
					405					410				415		
	Glu	Glu	Ala	Gln	Ala	Phe	Ile	Glu	Arg	Tyr	Phe	Gln	Ser	Phe	Pro	Lys
				420					425					430		
20	Val	Arg	Ala	Trp	Ile	Glu	Lys	Thr	Leu	Glu	Glu	Gly	Arg	Arg	Arg	Gly
			435					440					445			
	Tyr	Val	Glu	Thr	Leu	Phe	Gly	Arg	Arg	Arg	Tyr	Val	Pro	Asp	Leu	Glu
25		450					455					460				
	Ala	Arg	Val	Lys	Ser	Val	Arg	Glu	Ala	Ala	Glu	Arg	Met	Ala	Phe	Asn
	465					470					475				480	
30	Met	Pro	Val	Gln	Gly	Thr	Ala	Ala	Asp	Leu	Met	Lys	Leu	Ala	Met	Val
				485						490				495		
	Lys	Leu	Phe	Pro	Arg	Leu	Glu	Glu	Met	Gly	Ala	Arg	Met	Leu	Leu	Gln
				500					505					510		
35	Val	His	Asp	Glu	Leu	Val	Leu	Glu	Ala	Pro	Lys	Glu	Arg	Ala	Glu	Ala
			515					520					525			
	Val	Ala	Arg	Leu	Ala	Lys	Glu	Val	Met	Glu	Gly	Val	Tyr	Pro	Leu	Ala
40		530					535					540				
	Val	Pro	Leu	Glu	Val	Glu	Val	Gly	Ile	Gly	Glu	Asp	Trp	Leu	Ser	Ala

```

545
350
555
560

Lys Glu

5

<210> 8
<211> 958
<212> PRT
10 <213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Pho/Taq
      Chimeric polymerase

15
<400> 8
Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Asp Gly Lys Pro Ile Ile
  1              5              10              15

20 Arg Ile Phe Lys Lys Glu Asn Gly Glu Phe Lys Val Glu Tyr Asp Arg
      20              25              30

Asn Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Ala Ile
      35              40              45

25 Asp Glu Ile Lys Lys Ile Thr Ala Gln Arg His Gly Lys Val Val Arg
      50              55              60

Ile Val Glu Thr Glu Lys Ile Gln Arg Lys Phe Leu Gly Arg Pro Ile
30  65              70              75              80

Glu Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Ala Ile
      85              90              95

35 Arg Asp Lys Ile Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr
      100              105              110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Thr Pro
      115              120              125

40 Met Glu Gly Asn Glu Lys Leu Thr Phe Leu Ala Val Asp Ile Glu Thr
      130              135              140

```

Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Val Ile Met Ile  
 145 150 155 160

5 Ser Tyr Ala Asp Glu Glu Gly Ala Lys Val Ile Thr Trp Lys Lys Ile  
 165 170 175

Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys  
 180 185 190

10 Arg Leu Ile Arg Val Ile Lys Glu Lys Asp Pro Asp Val Ile Ile Thr  
 195 200 205

Tyr Asn Gly Asp Asn Phe Asp Phe Pro Tyr Leu Leu Lys Arg Ala Glu  
 15 210 215 220

Lys Leu Gly Ile Lys Leu Leu Leu Gly Arg Asp Asn Ser Glu Pro Lys  
 225 230 235 240

20 Met Gln Lys Met Gly Asp Ser Leu Ala Val Glu Ile Lys Gly Arg Ile  
 245 250 255

His Phe Asp Leu Phe Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr  
 260 265 270

25 Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu  
 275 280 285

Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Thr Gly Glu Gly  
 30 290 295 300

Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr  
 305 310 315 320

35 Glu Leu Gly Arg Glu Phe Phe Pro Met Glu Ala Gln Leu Ala Arg Leu  
 325 330 335

Val Gly Gln Pro Val Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu  
 340 345 350

40 Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala  
 355 360 365



Pro Asn Lys Pro Asp Glu Lys Glu Tyr Glu Arg Arg Leu Arg Glu Ser  
 370 375 380

5 Tyr Glu Gly Gly Tyr Val Lys Glu Pro Glu Lys Gly Ala Phe Leu Glu  
 385 390 395 400

Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu Glu Ser  
 405 410 415

10 Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly Ala Phe  
 420 425 430

Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp Leu Leu  
 15 435 440 445

Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro Glu Pro  
 450 455 460

20 Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu Ala Lys  
 465 470 475 480

Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro Pro Gly  
 485 490 495

25 Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn Thr Thr  
 500 505 510

Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu Glu Ala  
 30 515 520 525

Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu Trp Gly  
 530 535 540

35 Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu Val Glu  
 545 550 555 560

Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly Val Arg  
 565 570 575

40 Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala Glu Glu  
 580 585 590

Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His Pro Phe  
 595 600 605

5 Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp Glu Leu  
 610 615 620

Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg Ser Thr  
 625 630 635 640

10 Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile Val Glu  
 645 650 655

15 Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr Tyr Ile  
 660 665 670

Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu His Thr  
 675 680 685

20 Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser Ser Asp  
 690 695 700

Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln Arg Ile  
 705 710 715 720

25 Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala Leu Asp  
 725 730 735

30 Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly Asp Glu  
 740 745 750

Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr Glu Thr  
 755 760 765

35 Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro Leu Met  
 770 775 780

Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly Met Ser  
 785 790 795 800

40 Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu Ala Gln  
 805 810 815

Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg Ala Trp  
820 825 830

5 Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val Glu Thr  
835 840 845

Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg Val Lys  
850 855 860

10 Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro Val Gln  
865 870 875 880

Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu Phe Pro  
15 885 890 895

Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His Asp Glu  
900 905 910

20 Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala Arg Leu  
915 920 925

Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro Leu Glu  
930 935 940

25 Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu  
945 950 955

30 <210> 9  
<211> 958  
<212> PRT  
<213> Artificial Sequence

35 <220>  
<223> Description of Artificial Sequence: Pwo/Taq  
Chimeric polymerase

<400> 9

40 Met Ile Leu Asp Val Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile  
1 5 10 15

	Arg	Leu	Phe	Lys	Lys	Glu	Asn	Gly	Lys	Phe	Lys	Ile	Glu	His	Asp	Arg	
				20					25						30		
5	Thr	Phe	Arg	Pro	Tyr	Ile	Tyr	Ala	Leu	Leu	Arg	Asp	Asp	Ser	Lys	Ile	
			35					40					45				
	Glu	Glu	Val	Lys	Lys	Ile	Thr	Gly	Glu	Arg	His	Gly	Lys	Ile	Val	Arg	
		50					55					60					
10	Ile	Val	Asp	Val	Glu	Lys	Val	Glu	Lys	Lys	Phe	Leu	Gly	Lys	Pro	Ile	
	65					70					75					80	
	Thr	Val	Trp	Lys	Leu	Tyr	Leu	Glu	His	Pro	Gln	Asp	Val	Pro	Thr	Ile	
15					85					90					95		
	Arg	Glu	Lys	Val	Arg	Glu	His	Pro	Ala	Val	Val	Asp	Ile	Phe	Glu	Tyr	
				100					105					110			
20	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro	
			115					120					125				
	Met	Glu	Gly	Glu	Glu	Glu	Leu	Lys	Ile	Leu	Ala	Phe	Asp	Ile	Glu	Thr	
		130					135					140					
25	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Gly	Lys	Gly	Pro	Ile	Ile	Met	Ile	
	145					150					155					160	
	Ser	Tyr	Ala	Asp	Glu	Asn	Glu	Ala	Lys	Val	Ile	Thr	Trp	Lys	Asn	Ile	
30					165					170					175		
	Asp	Leu	Pro	Tyr	Val	Glu	Val	Val	Ser	Ser	Glu	Arg	Glu	Met	Ile	Lys	
				180					185					190			
35	Arg	Phe	Leu	Arg	Ile	Ile	Arg	Glu	Lys	Asp	Pro	Asp	Ile	Ile	Val	Thr	
			195					200					205				
	Tyr	Asn	Gly	Asp	Ser	Phe	Asp	Phe	Pro	Tyr	Leu	Ala	Lys	Arg	Ala	Glu	
		210					215					220					
40	Lys	Leu	Gly	Ile	Lys	Leu	Thr	Ile	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys	
	225					230					235					240	

	Met	Gln	Arg	Ile	Gly	Asp	Met	Thr	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile	
					245					250					255		
5	His	Phe	Asp	Leu	Tyr	His	Val	Ile	Thr	Arg	Thr	Ile	Asn	Leu	Pro	Thr	
				260					265					270			
	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Ile	Phe	Gly	Lys	Pro	Lys	Glu	
			275					280					285				
10	Lys	Val	Tyr	Ala	Asp	Glu	Ile	Ala	Lys	Ala	Trp	Glu	Ser	Gly	Glu	Asn	
		290					295					300					
	Leu	Glu	Arg	Val	Ala	Lys	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Ala	Thr	Tyr	
15	305					310					315					320	
	Glu	Leu	Gly	Lys	Glu	Phe	Leu	Pro	Met	Glu	Ile	Gln	Leu	Ser	Arg	Leu	
				325						330					335		
	Val	Gly	Gln	Pro	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu	
20				340					345					350			
	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Glu	Arg	Asn	Glu	Val	Ala	
		355						360					365				
25	Pro	Asn	Lys	Pro	Ser	Glu	Glu	Glu	Tyr	Gln	Arg	Arg	Leu	Arg	Glu	Ser	
		370					375					380					
	Tyr	Thr	Gly	Gly	Phe	Val	Lys	Glu	Pro	Glu	Lys	Gly	Ala	Phe	Leu	Glu	
30	385					390				395						400	
	Arg	Leu	Glu	Phe	Gly	Ser	Leu	Leu	His	Glu	Phe	Gly	Leu	Leu	Glu	Ser	
					405					410					415		
	Pro	Lys	Ala	Leu	Glu	Glu	Ala	Pro	Trp	Pro	Pro	Pro	Glu	Gly	Ala	Phe	
35				420					425					430			
	Val	Gly	Phe	Val	Leu	Ser	Arg	Lys	Glu	Pro	Met	Trp	Ala	Asp	Leu	Leu	
			435					440					445				
40	Ala	Leu	Ala	Ala	Ala	Arg	Gly	Gly	Arg	Val	His	Arg	Ala	Pro	Glu	Pro	
		450					455					460					

Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu Ala Lys  
 465 470 475 480

5 Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro Pro Gly  
 485 490 495

Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn Thr Thr  
 500 505 510

10 Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu Glu Ala  
 515 520 525

Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu Trp Gly  
 530 535 540

15 Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu Val Glu  
 545 550 555 560

Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly Val Arg  
 20 565 570 575

Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala Glu Glu  
 580 585 590

25 Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His Pro Phe  
 595 600 605

Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp Glu Leu  
 610 615 620

30 Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg Ser Thr  
 625 630 635 640

Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile Val Glu  
 35 645 650 655

Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr Tyr Ile  
 660 665 670

40 Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu His Thr  
 675 680 685

	Arg	Phe	Asn	Gln	Thr	Ala	Thr	Ala	Thr	Gly	Arg	Leu	Ser	Ser	Ser	Asp	
	690							695					700				
5	Pro	Asn	Leu	Gln	Asn	Ile	Pro	Val	Arg	Thr	Pro	Leu	Gly	Gln	Arg	Ile	
	705					710					715					720	
	Arg	Arg	Ala	Phe	Ile	Ala	Glu	Glu	Gly	Trp	Leu	Leu	Val	Ala	Leu	Asp	
					725					730						735	
10	Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Val	Leu	Ala	His	Leu	Ser	Gly	Asp	Glu	
				740						745					750		
	Asn	Leu	Ile	Arg	Val	Phe	Gln	Glu	Gly	Arg	Asp	Ile	His	Thr	Glu	Thr	
			755					760						765			
15	Ala	Ser	Trp	Met	Phe	Gly	Val	Pro	Arg	Glu	Ala	Val	Asp	Pro	Leu	Met	
		770					775						780				
	Arg	Arg	Ala	Ala	Lys	Thr	Ile	Asn	Phe	Gly	Val	Leu	Tyr	Gly	Met	Ser	
20	785				790						795					800	
	Ala	His	Arg	Leu	Ser	Gln	Glu	Leu	Ala	Ile	Pro	Tyr	Glu	Glu	Ala	Gln	
				805						810						815	
25	Ala	Phe	Ile	Glu	Arg	Tyr	Phe	Gln	Ser	Phe	Pro	Lys	Val	Arg	Ala	Trp	
			820						825					830			
	Ile	Glu	Lys	Thr	Leu	Glu	Glu	Gly	Arg	Arg	Arg	Gly	Tyr	Val	Glu	Thr	
			835					840					845				
30	Leu	Phe	Gly	Arg	Arg	Arg	Tyr	Val	Pro	Asp	Leu	Glu	Ala	Arg	Val	Lys	
		850					855					860					
	Ser	Val	Arg	Glu	Ala	Ala	Glu	Arg	Met	Ala	Phe	Asn	Met	Pro	Val	Gln	
35	865				870						875					880	
	Gly	Thr	Ala	Ala	Asp	Leu	Met	Lys	Leu	Ala	Met	Val	Lys	Leu	Phe	Pro	
				885						890						895	
40	Arg	Leu	Glu	Glu	Met	Gly	Ala	Arg	Met	Leu	Leu	Gln	Val	His	Asp	Glu	
				900					905							910	

Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala Arg Leu  
 915 920 925

Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro Leu Glu  
 5 930 935 940

Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu  
 945 950 955

10

&lt;210&gt; 10

&lt;211&gt; 1060

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

15

&lt;220&gt;

<223> Description of Artificial Sequence: Sso/Taq  
 Chimeric polymerase

20 &lt;400&gt; 10

Met Thr Lys Gln Leu Thr Leu Phe Asp Ile Pro Ser Ser Lys Pro Ala  
 1 5 10 15

Lys Ser Glu Gln Asn Thr Gln Gln Ser Gln Gln Ser Ala Pro Val Glu  
 25 20 25 30

Glu Lys Lys Val Val Arg Arg Glu Trp Leu Glu Glu Ala Gln Glu Asn  
 35 40 45

Lys Ile Tyr Phe Leu Leu Gln Val Asp Tyr Asp Gly Lys Lys Gly Lys  
 50 55 60

Ala Val Cys Lys Leu Phe Asp Lys Glu Thr Gln Lys Ile Tyr Ala Leu  
 65 70 75 80

35

Tyr Asp Asn Thr Gly His Lys Pro Tyr Phe Leu Val Asp Leu Glu Pro  
 85 90 95

Asp Lys Val Gly Lys Ile Pro Lys Ile Val Arg Asp Pro Ser Phe Asp  
 40 100 105 110

His Ile Glu Thr Val Ser Lys Ile Asp Pro Tyr Thr Trp Asn Lys Phe



	115		120		125
	Lys Leu Thr Lys Ile Val Val Arg Asp Pro His Ala Val Arg Arg Leu				
	130		135		140
5	Arg Asn Asp Val Pro Lys Ala Tyr Glu Ala His Ile Lys Tyr Phe Asn				
	145		150		155 160
	Asn Tyr Met Tyr Asp Ile Gly Leu Ile Pro Gly Met Pro Tyr Val Val				
10		165		170	175
	Lys Asn Gly Lys Leu Glu Ser Val Tyr Leu Ser Leu Asp Glu Lys Asp				
		180		185	190
15	Val Glu Glu Ile Lys Lys Ala Phe Ala Asp Ser Asp Glu Met Thr Arg				
		195		200	205
	Gln Met Ala Val Asp Trp Leu Pro Ile Phe Glu Thr Glu Ile Pro Lys				
		210		215	220
20	Ile Lys Arg Val Ala Ile Asp Ile Glu Val Tyr Thr Pro Val Lys Gly				
		225		230	235 240
	Arg Ile Pro Asp Ser Gln Lys Ala Glu Phe Pro Ile Ile Ser Ile Ala				
25		245		250	255
	Leu Ala Gly Ser Asp Gly Leu Lys Lys Val Leu Val Leu Asn Arg Asn				
		260		265	270
30	Asp Val Asn Glu Gly Ser Val Lys Leu Asp Gly Ile Ser Val Glu Arg				
		275		280	285
	Phe Asn Thr Glu Tyr Glu Leu Leu Gly Arg Phe Phe Asp Ile Leu Leu				
		290		295	300
35	Glu Tyr Pro Ile Val Leu Thr Phe Asn Gly Asp Asp Phe Asp Leu Pro				
		305		310	315 320
	Tyr Ile Tyr Phe Arg Ala Leu Lys Leu Gly Tyr Phe Pro Glu Glu Ile				
40		325		330	335
	Pro Ile Asp Val Ala Gly Lys Asp Glu Ala Lys Tyr Leu Ala Gly Leu				

		340		345		350											
	His	Ile	Asp	Leu	Tyr	Lys	Phe	Phe	Phe	Asn	Lys	Ala	Val	Arg	Asn	Tyr	
			355						360					365			
5	Ala	Phe	Glu	Gly	Lys	Tyr	Asn	Glu	Tyr	Asn	Leu	Asp	Ala	Val	Ala	Lys	
			370				375					380					
	Ala	Leu	Leu	Gly	Thr	Ser	Lys	Val	Lys	Val	Asp	Thr	Leu	Ile	Ser	Phe	
10	385					390					395					400	
	Leu	Asp	Val	Glu	Lys	Leu	Ile	Glu	Tyr	Asn	Phe	Arg	Asp	Ala	Glu	Ile	
					405					410					415		
15	Thr	Leu	Gln	Leu	Thr	Thr	Phe	Asn	Asn	Asp	Leu	Thr	Met	Lys	Leu	Ile	
				420					425					430			
	Val	Leu	Phe	Ser	Arg	Ile	Ser	Arg	Leu	Gly	Ile	Glu	Glu	Leu	Thr	Arg	
			435					440					445				
20	Thr	Glu	Ile	Ser	Thr	Trp	Val	Lys	Asn	Leu	Tyr	Tyr	Trp	Glu	His	Arg	
		450					455					460					
	Lys	Arg	Asn	Trp	Leu	Ile	Pro	Leu	Lys	Glu	Glu	Ile	Leu	Ala	Lys	Ser	
25	465					470					475					480	
	Ser	Asn	Ile	Arg	Thr	Ser	Ala	Leu	Ile	Lys	Gly	Lys	Gly	Tyr	Lys	Gly	
					485					490					495		
30	Ala	Val	Val	Ile	Asp	Pro	Pro	Ala	Gly	Ile	Phe	Phe	Leu	Leu	His	Glu	
				500					505					510			
	Phe	Gly	Leu	Leu	Glu	Ser	Pro	Lys	Ala	Leu	Glu	Glu	Ala	Pro	Trp	Pro	
			515					520					525				
35	Pro	Pro	Glu	Gly	Ala	Phe	Val	Gly	Phe	Val	Leu	Ser	Arg	Lys	Glu	Pro	
		530					535					540					
	Met	Trp	Ala	Asp	Leu	Leu	Ala	Leu	Ala	Ala	Ala	Arg	Gly	Gly	Arg	Val	
40	545					550				555					560		
	His	Arg	Ala	Pro	Glu	Pro	Tyr	Lys	Ala	Leu	Arg	Asp	Leu	Lys	Glu	Ala	

	565	570	575
	Arg Gly Leu Leu Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly		
	580	585	590
5	Leu Gly Leu Pro Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu		
	595	600	605
	Asp Pro Ser Asn Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly		
10	610	615	620
	Glu Trp Thr Glu Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu		
	625	630	635 640
15	Phe Ala Asn Leu Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp		
	645	650	655
	Leu Tyr Arg Glu Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met		
	660	665	670
20	Glu Ala Thr Gly Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser		
	675	680	685
	Leu Glu Val Ala Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg		
25	690	695	700
	Leu Ala Gly His Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg		
	705	710	715 720
30	Val Leu Phe Asp Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys		
	725	730	735
	Thr Gly Lys Arg Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu		
	740	745	750
35	Ala His Pro Ile Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys		
	755	760	765
	Leu Lys Ser Thr Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg		
40	770	775	780
	Thr Gly Arg Leu His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly		

	785		790		795		800									
	Arg	Leu	Ser	Ser	Asp	Pro	Asn	Leu	Gln	Asn	Ile	Pro	Val	Arg	Thr	
				805					810					815		
5	Pro	Leu	Gly	Gln	Arg	Ile	Arg	Arg	Ala	Phe	Ile	Ala	Glu	Glu	Gly	Trp
				820					825					830		
	Leu	Leu	Val	Ala	Leu	Asp	Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Val	Leu	Ala
10			835					840					845			
	His	Leu	Ser	Gly	Asp	Glu	Asn	Leu	Ile	Arg	Val	Phe	Gln	Glu	Gly	Arg
		850					855					860				
15	Asp	Ile	His	Thr	Glu	Thr	Ala	Ser	Trp	Met	Phe	Gly	Val	Pro	Arg	Glu
	865					870					875					880
	Ala	Val	Asp	Pro	Leu	Met	Arg	Arg	Ala	Ala	Lys	Thr	Ile	Asn	Phe	Gly
					885					890						895
20	Val	Leu	Tyr	Gly	Met	Ser	Ala	His	Arg	Leu	Ser	Gln	Glu	Leu	Ala	Ile
				900					905						910	
	Pro	Tyr	Glu	Glu	Ala	Gln	Ala	Phe	Ile	Glu	Arg	Tyr	Phe	Gln	Ser	Phe
25			915					920					925			
	Pro	Lys	Val	Arg	Ala	Trp	Ile	Glu	Lys	Thr	Leu	Glu	Glu	Gly	Arg	Arg
		930					935						940			
30	Arg	Gly	Tyr	Val	Glu	Thr	Leu	Phe	Gly	Arg	Arg	Arg	Tyr	Val	Pro	Asp
	945					950					955					960
	Leu	Glu	Ala	Arg	Val	Lys	Ser	Val	Arg	Glu	Ala	Ala	Glu	Arg	Met	Ala
					965					970						975
35	Phe	Asn	Met	Pro	Val	Gln	Gly	Thr	Ala	Ala	Asp	Leu	Met	Lys	Leu	Ala
				980					985					990		
	Met	Val	Lys	Leu	Phe	Pro	Arg	Leu	Glu	Glu	Met	Gly	Ala	Arg	Met	Leu
40			995					1000					1005			
	Leu	Gln	Val	His	Asp	Glu	Leu	Val	Leu	Glu	Ala	Pro	Lys	Glu	Arg	Ala

	1010	1015	1020
	Glu Ala Val Ala Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro		
	1025	1030	1035 1040
5	Leu Ala Val Pro Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu		
	1045	1050	1055
	Ser Ala Lys Glu		
10	1060		
	<210> 11		
	<211> 983		
15	<212> PRT		
	<213> Artificial Sequence		
	<220>		
	<223> Description of Artificial Sequence: Pwo/Taq		
20	Chimeric polymerase		
	<400> 11		
	Met Ile Leu Asp Val Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val Ile		
	1 5 10 15		
25	Arg Leu Phe Lys Lys Glu Asn Gly Lys Phe Lys Ile Glu His Asp Arg		
	20 25 30		
	Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Lys Ile		
30	35 40 45		
	Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val Arg		
	50 55 60		
35	Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro Ile		
	65 70 75 80		
	Thr Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Thr Ile		
	85 90 95		
40	Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr		
	100 105 110		

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro  
 115 120 125

5 Met Glu Gly Glu Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu Thr  
 130 135 140

Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile  
 145 150 155 160

10 Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn Ile  
 165 170 175

Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys  
 15 180 185 190

Arg Phe Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val Thr  
 195 200 205

20 Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala Glu  
 210 215 220

Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys  
 225 230 235 240

25 Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile  
 245 250 255

His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro Thr  
 30 260 265 270

Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu  
 275 280 285

35 Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu Asn  
 290 295 300

Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr  
 305 310 315 320

40 Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg Leu  
 325 330 335

	Val	Gly	Gln	Pro	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu	
				340					345					350			
5	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Glu	Arg	Asn	Glu	Val	Ala	
			355					360					365				
	Pro	Asn	Lys	Pro	Ser	Glu	Glu	Glu	Tyr	Gln	Arg	Arg	Leu	Arg	Glu	Ser	
		370					375					380					
10	Tyr	Thr	Gly	Gly	Phe	Val	Lys	Glu	Pro	Glu	Lys	Gly	Leu	Trp	Glu	Asn	
	385					390					395					400	
	Ile	Val	Tyr	Leu	Asp	Phe	Arg	Ala	Leu	Tyr	Pro	Ser	Ile	Ile	Ile	Thr	
15				405						410					415		
	His	Asn	Val	Ser	Pro	Ala	Phe	Leu	Glu	Arg	Leu	Glu	Phe	Gly	Ser	Leu	
			420						425					430			
20	Leu	His	Glu	Phe	Gly	Leu	Leu	Glu	Ser	Pro	Lys	Ala	Leu	Glu	Glu	Ala	
			435					440					445				
	Pro	Trp	Pro	Pro	Pro	Glu	Gly	Ala	Phe	Val	Gly	Phe	Val	Leu	Ser	Arg	
		450					455					460					
25	Lys	Glu	Pro	Met	Trp	Ala	Asp	Leu	Leu	Ala	Leu	Ala	Ala	Ala	Arg	Gly	
	465					470					475					480	
	Gly	Arg	Val	His	Arg	Ala	Pro	Glu	Pro	Tyr	Lys	Ala	Leu	Arg	Asp	Leu	
30				485						490					495		
	Lys	Glu	Ala	Arg	Gly	Leu	Leu	Ala	Lys	Asp	Leu	Ser	Val	Leu	Ala	Leu	
			500						505					510			
35	Arg	Glu	Gly	Leu	Gly	Leu	Pro	Pro	Gly	Asp	Asp	Pro	Met	Leu	Leu	Ala	
		515						520					525				
	Tyr	Leu	Leu	Asp	Pro	Ser	Asn	Thr	Thr	Pro	Glu	Gly	Val	Ala	Arg	Arg	
	530						535					540					
40	Tyr	Gly	Gly	Glu	Trp	Thr	Glu	Glu	Ala	Gly	Glu	Arg	Ala	Ala	Leu	Ser	
	545					550					555					560	

Glu Arg Leu Phe Ala Asn Leu Trp Gly Arg Leu Glu Gly Glu Glu Arg  
 565 570 575

5 Leu Leu Trp Leu Tyr Arg Glu Val Glu Arg Pro Leu Ser Ala Val Leu  
 580 585 590

Ala His Met Glu Ala Thr Gly Val Arg Leu Asp Val Ala Tyr Leu Arg  
 595 600 605

10 Ala Leu Ser Leu Glu Val Ala Glu Glu Ile Ala Arg Leu Glu Ala Glu  
 610 615 620

Val Phe Arg Leu Ala Gly His Pro Phe Asn Leu Asn Ser Arg Asp Gln  
 15 625 630 635 640

Leu Glu Arg Val Leu Phe Asp Glu Leu Gly Leu Pro Ala Ile Gly Lys  
 645 650 655

20 Thr Glu Lys Thr Gly Lys Arg Ser Thr Ser Ala Ala Val Leu Glu Ala  
 660 665 670

Leu Arg Glu Ala His Pro Ile Val Glu Lys Ile Leu Gln Tyr Arg Glu  
 675 680 685

25 Leu Thr Lys Leu Lys Ser Thr Tyr Ile Asp Pro Leu Pro Asp Leu Ile  
 690 695 700

His Pro Arg Thr Gly Arg Leu His Thr Arg Phe Asn Gln Thr Ala Thr  
 30 705 710 715 720

Ala Thr Gly Arg Leu Ser Ser Ser Asp Pro Asn Leu Gln Asn Ile Pro  
 725 730 735

35 Val Arg Thr Pro Leu Gly Gln Arg Ile Arg Arg Ala Phe Ile Ala Glu  
 740 745 750

Glu Gly Trp Leu Leu Val Ala Leu Asp Tyr Ser Gln Ile Glu Leu Arg  
 755 760 765

40 Val Leu Ala His Leu Ser Gly Asp Glu Asn Leu Ile Arg Val Phe Gln  
 770 775 780



Glu Gly Arg Asp Ile His Thr Glu Thr Ala Ser Trp Met Phe Gly Val  
 785 790 795 800

5 Pro Arg Glu Ala Val Asp Pro Leu Met Arg Arg Ala Ala Lys Thr Ile  
 805 810 815

Asn Phe Gly Val Leu Tyr Gly Met Ser Ala His Arg Leu Ser Gln Glu  
 820 825 830

10 Leu Ala Ile Pro Tyr Glu Glu Ala Gln Ala Phe Ile Glu Arg Tyr Phe  
 835 840 845

Gln Ser Phe Pro Lys Val Arg Ala Trp Ile Glu Lys Thr Leu Glu Glu  
 15 850 855 860

Gly Arg Arg Arg Gly Tyr Val Glu Thr Leu Phe Gly Arg Arg Arg Tyr  
 865 870 875 880

20 Val Pro Asp Leu Glu Ala Arg Val Lys Ser Val Arg Glu Ala Ala Glu  
 885 890 895

Arg Met Ala Phe Asn Met Pro Val Gln Gly Thr Ala Ala Asp Leu Met  
 900 905 910

25 Lys Leu Ala Met Val Lys Leu Phe Pro Arg Leu Glu Glu Met Gly Ala  
 915 920 925

Arg Met Leu Leu Gln Val His Asp Glu Leu Val Leu Glu Ala Pro Lys  
 30 930 935 940

Glu Arg Ala Glu Ala Val Ala Arg Leu Ala Lys Glu Val Met Glu Gly  
 945 950 955 960

35 Val Tyr Pro Leu Ala Val Pro Leu Glu Val Glu Val Gly Ile Gly Glu  
 965 970 975 ...

Asp Trp Leu Ser Ala Lys Glu  
 980

40

<210> 12

&lt;211&gt; 958

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

5 &lt;220&gt;

<223> Description of Artificial Sequence: Pho/Tth  
Chimeric polymerase

&lt;400&gt; 12

```

10 Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Asp Gly Lys Pro Ile Ile
    1             5             10             15

    Arg Ile Phe Lys Lys Glu Asn Gly Glu Phe Lys Val Glu Tyr Asp Arg
                20             25             30
15 Asn Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Ala Ile
    35             40             45

    Asp Glu Ile Lys Lys Ile Thr Ala Gln Arg His Gly Lys Val Val Arg
20     50             55             60

    Ile Val Glu Thr Glu Lys Ile Gln Arg Lys Phe Leu Gly Arg Pro Ile
    65             70             75             80

25 Glu Val Trp Lys Leu Tyr Leu Glu His Pro Gln Asp Val Pro Ala Ile
    85             90             95

    Arg Asp Lys Ile Arg Glu His Pro Ala Val Val Asp Ile Phe Glu Tyr
    100            105            110
30 Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Thr Pro
    115            120            125

    Met Glu Gly Asn Glu Lys Leu Thr Phe Leu Ala Val Asp Ile Glu Thr
35     130            135            140

    Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Val Ile Met Ile
    145            150            155            160

40 Ser Tyr Ala Asp Glu Glu Gly Ala Lys Val Ile Thr Trp Lys Lys Ile
    165            170            175

```

	Asp	Leu	Pro	Tyr	Val	Glu	Val	Val	Ser	Ser	Glu	Arg	Glu	Met	Ile	Lys	
						180				185					190		
5	Arg	Leu	Ile	Arg	Val	Ile	Lys	Glu	Lys	Asp	Pro	Asp	Val	Ile	Ile	Thr	
			195					200					205				
	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Pro	Tyr	Leu	Leu	Lys	Arg	Ala	Glu	
		210					215					220					
10	Lys	Leu	Gly	Ile	Lys	Leu	Leu	Leu	Gly	Arg	Asp	Asn	Ser	Glu	Pro	Lys	
	225					230					235					240	
	Met	Gln	Lys	Met	Gly	Asp	Ser	Leu	Ala	Val	Glu	Ile	Lys	Gly	Arg	Ile	
					245					250					255		
15	His	Phe	Asp	Leu	Phe	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr	
				260					265					270			
	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Ile	Phe	Gly	Lys	Pro	Lys	Glu	
20			275					280					285				
	Lys	Val	Tyr	Ala	Asp	Glu	Ile	Ala	Lys	Ala	Trp	Glu	Thr	Gly	Glu	Gly	
		290					295					300					
25	Leu	Glu	Arg	Val	Ala	Lys	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr	
	305					310					315					320	
	Glu	Leu	Gly	Arg	Glu	Phe	Phe	Pro	Met	Glu	Ala	Gln	Leu	Ala	Arg	Leu	
					325					330					335		
30	Val	Gly	Gln	Pro	Val	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu	
				340					345					350			
	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Glu	Arg	Asn	Glu	Leu	Ala	
35			355					360					365				
	Pro	Asn	Lys	Pro	Asp	Glu	Lys	Glu	Tyr	Glu	Arg	Arg	Leu	Arg	Glu	Ser	
		370					375					380					
40	Tyr	Glu	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Lys	Gly	Ala	Phe	Leu	Glu	
	385					390					395					400	

	Arg	Leu	Glu	Phe	Gly	Ser	Leu	Leu	His	Glu	Phe	Gly	Leu	Leu	Glu	Ala	
					405					410					415		
5	Pro	Ala	Pro	Leu	Glu	Glu	Ala	Pro	Trp	Pro	Pro	Pro	Glu	Gly	Ala	Phe	
				420					425					430			
	Val	Gly	Phe	Val	Leu	Ser	Arg	Pro	Glu	Pro	Met	Trp	Ala	Glu	Leu	Lys	
			435					440					445				
10	Ala	Leu	Ala	Ala	Cys	Arg	Asp	Gly	Arg	Val	His	Arg	Ala	Ala	Asp	Pro	
		450					455					460					
	Leu	Ala	Gly	Leu	Lys	Asp	Leu	Lys	Glu	Val	Arg	Gly	Leu	Leu	Ala	Lys	
	465				470						475					480	
15	Asp	Leu	Ala	Val	Leu	Ala	Ser	Arg	Glu	Gly	Leu	Asp	Leu	Val	Pro	Gly	
					485					490					495		
	Asp	Asp	Pro	Met	Leu	Leu	Ala	Tyr	Leu	Leu	Asp	Pro	Ser	Asn	Thr	Thr	
20				500					505					510			
	Pro	Glu	Gly	Val	Ala	Arg	Arg	Tyr	Gly	Gly	Glu	Trp	Thr	Glu	Asp	Ala	
			515					520					525				
25	Ala	His	Arg	Ala	Leu	Leu	Ser	Glu	Arg	Leu	His	Arg	Asn	Leu	Leu	Lys	
		530					535					540					
	Arg	Leu	Glu	Gly	Glu	Glu	Lys	Leu	Leu	Trp	Leu	Tyr	His	Glu	Val	Glu	
	545				550					555						560	
30	Lys	Pro	Leu	Ser	Arg	Val	Leu	Ala	His	Met	Glu	Ala	Thr	Gly	Val	Arg	
					565					570					575		
	Arg	Asp	Val	Ala	Tyr	Leu	Gln	Ala	Leu	Ser	Leu	Glu	Leu	Ala	Glu	Glu	
35			580					585						590			
	Ile	Arg	Arg	Leu	Glu	Glu	Glu	Val	Phe	Arg	Leu	Ala	Gly	His	Pro	Phe	
			595					600					605				
40	Asn	Leu	Asn	Ser	Arg	Asp	Gln	Leu	Glu	Arg	Val	Leu	Phe	Asp	Glu	Leu	
		610					615					620					

	Arg	Leu	Pro	Ala	Leu	Gly	Lys	Thr	Gln	Lys	Thr	Gly	Lys	Arg	Ser	Thr	
	625					630					635					640	
5	Ser	Ala	Ala	Val	Leu	Glu	Ala	Leu	Arg	Glu	Ala	His	Pro	Ile	Val	Glu	
					645					650					655		
	Lys	Ile	Leu	Gln	His	Arg	Glu	Leu	Thr	Lys	Leu	Lys	Asn	Thr	Tyr	Val	
				660					665					670			
10	Asp	Pro	Leu	Pro	Ser	Leu	Val	His	Pro	Arg	Thr	Gly	Arg	Leu	His	Thr	
			675					680					685				
	Arg	Phe	Asn	Gln	Thr	Ala	Thr	Ala	Thr	Gly	Arg	Leu	Ser	Ser	Ser	Asp	
		690					695					700					
15	Pro	Asn	Leu	Gln	Asn	Ile	Pro	Val	Arg	Thr	Pro	Leu	Gly	Gln	Arg	Ile	
	705					710					715					720	
	Arg	Arg	Ala	Phe	Val	Ala	Glu	Ala	Gly	Trp	Ala	Leu	Val	Ala	Leu	Asp	
20					725					730					735		
	Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Val	Leu	Ala	His	Leu	Ser	Gly	Asp	Glu	
				740					745					750			
25	Asn	Leu	Ile	Arg	Val	Phe	Gln	Glu	Gly	Lys	Asp	Ile	His	Thr	Gln	Thr	
		755						760					765				
	Ala	Ser	Trp	Met	Phe	Gly	Val	Pro	Pro	Glu	Ala	Val	Asp	Pro	Leu	Met	
		770					775						780				
30	Arg	Arg	Ala	Ala	Lys	Thr	Val	Asn	Phe	Gly	Val	Leu	Tyr	Gly	Met	Ser	
	785					790					795					800	
	Ala	His	Arg	Leu	Ser	Gln	Glu	Leu	Ala	Ile	Pro	Tyr	Glu	Glu	Ala	Val	
35					805					810					815		
	Ala	Phe	Ile	Glu	Arg	Tyr	Phe	Gln	Ser	Phe	Pro	Lys	Val	Arg	Ala	Trp	
				820					825					830			
40	Ile	Glu	Lys	Thr	Leu	Glu	Glu	Gly	Arg	Lys	Arg	Gly	Tyr	Val	Glu	Thr	
		835						840					845				

Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Asn Ala Arg Val Lys  
850 855 860

5 Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro Val Gln  
865 870 875 880

Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu Phe Pro  
885 890 895

10 Arg Leu Arg Glu Met Gly Ala Arg Met Leu Leu Gln Val His Asp Glu  
900 905 910

Leu Leu Leu Glu Ala Pro Gln Ala Arg Ala Glu Glu Val Ala Ala Leu  
915 920 925

15 Ala Lys Glu Ala Met Glu Lys Ala Tyr Pro Leu Ala Val Pro Leu Glu  
930 935 940

Val Glu Val Gly Met Gly Glu Asp Trp Leu Ser Ala Lys Gly  
20 945 950 955

<210> 13  
<211> 30  
25 <212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Primer  
30 sequence

<400> 13  
tttcccagtc acgacgttgt aaaacgacgg  
30

35

<210> 14  
<211> 19  
<212> DNA  
40 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer  
sequence

<400> 14

5 gcaccccgct tgggcagag  
19

<210> 15

10 <211> 20

<212> DNA

<213> Artificial Sequence.

<220>

15 <223> Description of Artificial Sequence: Primer  
sequence

<400> 15

tcccggcccct cctggaagac  
20 20

<210> 16

<211> 395

25 <212> PRT

<213> Thermococcus pacificus

<400> 16

Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Asp Gly Lys Pro Val Ile  
30 1 5 10 15

Arg Ile Phe Arg Lys Glu Lys Gly Glu Phe Lys Ile Glu Tyr Asp Arg  
20 25 30

35 Asn Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile  
35 40 45

Glu Asp Val Lys Lys Ile Thr Ala Glu Arg His Gly Thr Thr Val Arg  
50 55 60

40 Val Val Arg Ala Glu Lys Val Lys Lys Lys Phe Leu Gly Arg Pro Ile  
65 70 75 80

Glu Val Trp Lys Leu Tyr Phe Thr His Pro Gln Asp Val Pro Ala Ile  
                                     85                                    90                                    95

5 Arg Asp Lys Ile Arg Glu His Pro Ala Val Val Asp Ile Tyr Glu Tyr  
                                     100                                    105                                    110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro  
                                     115                                    120                                    125

10 Met Glu Gly Asp Glu Glu Leu Lys Met Leu Ala Phe Asp Ile Glu Thr  
                                     130                                    135                                    140

Leu Tyr His Glu Gly Glu Glu Phe Ala Glu Gly Pro Ile Leu Met Ile  
 15 145                                    150                                    155                                    160

Ser Tyr Ala Asp Glu Glu Gly Ala Arg Val Ile Thr Trp Lys Asn Ile  
                                     165                                    170                                    175

20 Asp Leu Pro Tyr Val Asp Val Val Ser Thr Glu Lys Glu Met Ile Lys  
                                     180                                    185                                    190

Arg Phe Leu Arg Val Ile Lys Glu Lys Asp Pro Asp Val Leu Ile Thr  
                                     195                                    200                                    205

25 Tyr Asn Gly Asp Asn Phe Asp Phe Ala Tyr Leu Lys Lys Arg Ser Glu  
                                     210                                    215                                    220

Lys Leu Gly Val Lys Phe Ile Leu Gly Arg Asp Gly Ser Glu Pro Lys  
 30 225                                    230                                    235                                    240

Ile Gln Arg Met Gly Asp Arg Phe Ala Val Glu Val Lys Gly Arg Ile  
                                     245                                    250                                    255

35 His Phe Asp Leu Tyr Pro Val Ile Arg Arg Thr Ile Asn Leu Pro Thr  
                                     260                                    265                                    270

Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Gln Pro Lys Glu  
                                     275                                    280                                    285

40 Lys Val Tyr Ala Glu Glu Ile Thr Gln Ala Trp Glu Thr Gly Glu Gly  
                                     290                                    295                                    300



Leu Glu Arg Val Ala Arg Tyr Ser Met Glu Asp Ala Lys Val Thr Tyr  
 305 310 315 320

5 Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Gln Leu Ser Arg Leu  
 325 330 335

Val Gly Gln Ser Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn Leu  
 340 345 350

10 Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Leu Ala  
 355 360 365

Pro Asn Lys Pro Asp Glu Lys Glu Leu Ala Arg Arg Arg Glu Ser Tyr  
 15 370 375 380

Ala Gly Gly Tyr Val Lys Glu Pro Glu Lys Gly  
 385 390 395

20 <210> 17  
 <211> 957  
 <212> PRT  
 <213> Artificial Sequence

25 <220>  
 <223> Description of Artificial Sequence: Tpac/Taq  
 Chimeric polymerase

30 <400> 17  
 Met Ile Leu Asp Ala Asp Tyr Ile Thr Glu Asp Gly Lys Pro Val Ile  
 1 5 10 15

Arg Ile Phe Arg Lys Glu Lys Gly Glu Phe Lys Ile Glu Tyr Asp Arg  
 35 20 25 30

Asn Phe Glu Pro Tyr Phe Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile  
 35 40 45

40 Glu Asp Val Lys Lys Ile Thr Ala Glu Arg His Gly Thr Thr Val Arg  
 50 55 60

	Val	Val	Arg	Ala	Glu	Lys	Val	Lys	Lys	Lys	Phe	Leu	Gly	Arg	Pro	Ile	
	65						70				75					80	
5	Glu	Val	Trp	Lys	Leu	Tyr	Phe	Thr	His	Pro	Gln	Asp	Val	Pro	Ala	Ile	
					85					90					95		
	Arg	Asp	Lys	Ile	Arg	Glu	His	Pro	Ala	Val	Val	Asp	Ile	Tyr	Glu	Tyr	
				100					105					110			
10	Asp	Ile	Pro	Phe	Ala	Lys	Arg	Tyr	Leu	Ile	Asp	Lys	Gly	Leu	Ile	Pro	
			115					120					125				
	Met	Glu	Gly	Asp	Glu	Glu	Leu	Lys	Met	Leu	Ala	Phe	Asp	Ile	Glu	Thr	
	130						135					140					
15	Leu	Tyr	His	Glu	Gly	Glu	Glu	Phe	Ala	Glu	Gly	Pro	Ile	Leu	Met	Ile	
	145					150					155					160	
	Ser	Tyr	Ala	Asp	Glu	Glu	Gly	Ala	Arg	Val	Ile	Thr	Trp	Lys	Asn	Ile	
20					165					170					175		
	Asp	Leu	Pro	Tyr	Val	Asp	Val	Val	Ser	Thr	Glu	Lys	Glu	Met	Ile	Lys	
				180					185					190			
25	Arg	Phe	Leu	Arg	Val	Ile	Lys	Glu	Lys	Asp	Pro	Asp	Val	Leu	Ile	Thr	
			195					200					205				
	Tyr	Asn	Gly	Asp	Asn	Phe	Asp	Phe	Ala	Tyr	Leu	Lys	Lys	Arg	Ser	Glu	
	210					215						220					
30	Lys	Leu	Gly	Val	Lys	Phe	Ile	Leu	Gly	Arg	Asp	Gly	Ser	Glu	Pro	Lys	
	225					230					235					240	
	Ile	Gln	Arg	Met	Gly	Asp	Arg	Phe	Ala	Val	Glu	Val	Lys	Gly	Arg	Ile	
35					245					250					255		
	His	Phe	Asp	Leu	Tyr	Pro	Val	Ile	Arg	Arg	Thr	Ile	Asn	Leu	Pro	Thr	
				260					265					270			
40	Tyr	Thr	Leu	Glu	Ala	Val	Tyr	Glu	Ala	Ile	Phe	Gly	Gln	Pro	Lys	Glu	
			275					280					285				

	Lys	Val	Tyr	Ala	Glu	Glu	Ile	Thr	Gln	Ala	Trp	Glu	Thr	Gly	Glu	Gly	
	290						295					300					
5	Leu	Glu	Arg	Val	Ala	Arg	Tyr	Ser	Met	Glu	Asp	Ala	Lys	Val	Thr	Tyr	
	305					310					315					320	
	Glu	Leu	Gly	Lys	Glu	Phe	Phe	Pro	Met	Glu	Ala	Gln	Leu	Ser	Arg	Leu	
					325					330					335		
10	Val	Gly	Gln	Ser	Leu	Trp	Asp	Val	Ser	Arg	Ser	Ser	Thr	Gly	Asn	Leu	
				340					345					350			
	Val	Glu	Trp	Phe	Leu	Leu	Arg	Lys	Ala	Tyr	Glu	Arg	Asn	Glu	Leu	Ala	
		355					360						365				
15	Pro	Asn	Lys	Pro	Asp	Glu	Lys	Glu	Leu	Ala	Arg	Arg	Arg	Glu	Ser	Tyr	
	370						375					380					
	Ala	Gly	Gly	Tyr	Val	Lys	Glu	Pro	Glu	Lys	Gly	Ala	Phe	Leu	Glu	Arg	
20	385					390					395					400	
	Leu	Glu	Phe	Gly	Ser	Leu	Leu	His	Glu	Phe	Gly	Leu	Leu	Glu	Ser	Pro	
					405					410					415		
25	Lys	Ala	Leu	Glu	Glu	Ala	Pro	Trp	Pro	Pro	Pro	Glu	Gly	Ala	Phe	Val	
				420					425					430			
	Gly	Phe	Val	Leu	Ser	Arg	Lys	Glu	Pro	Met	Trp	Ala	Asp	Leu	Leu	Ala	
		435						440					445				
30	Leu	Ala	Ala	Ala	Arg	Gly	Gly	Arg	Val	His	Arg	Ala	Pro	Glu	Pro	Tyr	
	450						455					460					
	Lys	Ala	Leu	Arg	Asp	Leu	Lys	Glu	Ala	Arg	Gly	Leu	Leu	Ala	Lys	Asp	
35	465					470					475					480	
	Leu	Ser	Val	Leu	Ala	Leu	Arg	Glu	Gly	Leu	Gly	Leu	Pro	Pro	Gly	Asp	
					485					490					495		
40	Asp	Pro	Met	Leu	Leu	Ala	Tyr	Leu	Leu	Asp	Pro	Ser	Asn	Thr	Thr	Pro	
				500						505				510			

	Glu	Gly	Val	Ala	Arg	Arg	Tyr	Gly	Gly	Glu	Trp	Thr	Glu	Glu	Ala	Gly	
			515					520					525				
5	Glu	Arg	Ala	Ala	Leu	Ser	Glu	Arg	Leu	Phe	Ala	Asn	Leu	Trp	Gly	Arg	
		530					535					540					
	Leu	Glu	Gly	Glu	Glu	Arg	Leu	Leu	Trp	Leu	Tyr	Arg	Glu	Val	Glu	Arg	
	545					550					555					560	
10	Pro	Leu	Ser	Ala	Val	Leu	Ala	His	Met	Glu	Ala	Thr	Gly	Val	Arg	Leu	
					565					570					575		
	Asp	Val	Ala	Tyr	Leu	Arg	Ala	Leu	Ser	Leu	Glu	Val	Ala	Glu	Glu	Ile	
				580					585					590			
15	Ala	Arg	Leu	Glu	Ala	Glu	Val	Phe	Arg	Leu	Ala	Gly	His	Pro	Phe	Asn	
			595					600					605				
	Leu	Asn	Ser	Arg	Asp	Gln	Leu	Glu	Arg	Val	Leu	Phe	Asp	Glu	Leu	Gly	
20		610					615					620					
	Leu	Pro	Ala	Ile	Gly	Lys	Thr	Glu	Lys	Thr	Gly	Lys	Arg	Ser	Thr	Ser	
	625					630					635					640	
25	Ala	Ala	Val	Leu	Glu	Ala	Leu	Arg	Glu	Ala	His	Pro	Ile	Val	Glu	Lys	
					645					650					655		
	Ile	Leu	Gln	Tyr	Arg	Glu	Leu	Thr	Lys	Leu	Lys	Ser	Thr	Tyr	Ile	Asp	
				660					665					670			
30	Pro	Leu	Pro	Asp	Leu	Ile	His	Pro	Arg	Thr	Gly	Arg	Leu	His	Thr	Arg	
			675					680					685				
	Phe	Asn	Gln	Thr	Ala	Thr	Ala	Thr	Gly	Arg	Leu	Ser	Ser	Ser	Asp	Pro	
35		690					695					700					
	Asn	Leu	Gln	Asn	Ile	Pro	Val	Arg	Thr	Pro	Leu	Gly	Gln	Arg	Ile	Arg	
	705					710					715					720	
40	Arg	Ala	Phe	Ile	Ala	Glu	Glu	Gly	Trp	Leu	Leu	Val	Ala	Leu	Asp	Tyr	
					725					730					735		

	Ser	Gln	Ile	Glu	Leu	Arg	Val	Leu	Ala	His	Leu	Ser	Gly	Asp	Glu	Asn	
				740					745					750			
5	Leu	Ile	Arg	Val	Phe	Gln	Glu	Gly	Arg	Asp	Ile	His	Thr	Glu	Thr	Ala	
			755					760					765				
	Ser	Trp	Met	Phe	Gly	Val	Pro	Arg	Glu	Ala	Val	Asp	Pro	Leu	Met	Arg	
			770				775					780					
10	Arg	Ala	Ala	Lys	Thr	Ile	Asn	Phe	Gly	Val	Leu	Tyr	Gly	Met	Ser	Ala	
						790					795					800	
	His	Arg	Leu	Ser	Gln	Glu	Leu	Ala	Ile	Pro	Tyr	Glu	Glu	Ala	Gln	Ala	
					805					810					815		
15	Phe	Ile	Glu	Arg	Tyr	Phe	Gln	Ser	Phe	Pro	Lys	Val	Arg	Ala	Trp	Ile	
				820					825					830			
	Glu	Lys	Thr	Leu	Glu	Glu	Gly	Arg	Arg	Arg	Gly	Tyr	Val	Glu	Thr	Leu	
20			835					840					845				
	Phe	Gly	Arg	Arg	Arg	Tyr	Val	Pro	Asp	Leu	Glu	Ala	Arg	Val	Lys	Ser	
			850				855					860					
25	Val	Arg	Glu	Ala	Ala	Glu	Arg	Met	Ala	Phe	Asn	Met	Pro	Val	Gln	Gly	
						870					875					880	
	Thr	Ala	Ala	Asp	Leu	Met	Lys	Leu	Ala	Met	Val	Lys	Leu	Phe	Pro	Arg	
					885					890					895		
30	Leu	Glu	Glu	Met	Gly	Ala	Arg	Met	Leu	Leu	Gln	Val	His	Asp	Glu	Leu	
				900					905					910			
	Val	Leu	Glu	Ala	Pro	Lys	Glu	Arg	Ala	Glu	Ala	Val	Ala	Arg	Leu	Ala	
35			915					920					925				
	Lys	Glu	Val	Met	Glu	Gly	Val	Tyr	Pro	Leu	Ala	Val	Pro	Leu	Glu	Val	
			930				935					940					
40	Glu	Val	Gly	Ile	Gly	Glu	Asp	Trp	Leu	Ser	Ala	Lys	Glu				
					945		950				955						

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
23 August 2001 (23.08.2001)

PCT

(10) International Publication Number  
**WO 01/61015 A3**

(51) International Patent Classification<sup>7</sup>: C12N 15/54,  
15/62, 15/70, 9/12, 1/21, C12Q 1/68, C12P 19/34

(21) International Application Number: PCT/EP01/01790

(22) International Filing Date: 16 February 2001 (16.02.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
09/506,153 17 February 2000 (17.02.2000) US

(63) Related by continuation (CON) or continuation-in-part  
(CIP) to earlier application:  
US 09/506,153 (CIP)  
Filed on 17 February 2000 (17.02.2000)

(71) Applicant (for all designated States except US): QIAGEN  
GMBH [DE/DE]; Max-Volmer-Strasse 4, 40724 Hilden  
(DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): LÖFFERT, Dirk

[DE/DE]; Bahlenstrasse 8, 40589 Düsseldorf (DE). MIS-  
SEL, Andreas [DE/DE]; Kirchfeldstrasse 93, 40215 Düs-  
seldorf (DE). KANG, Jie [DE/DE]; Meisenweg 25, 40822  
Mettmann (DE).

(74) Common Representative: QIAGEN GMBH;  
Max-Volmer-Strasse 4, 40724 Hilden (DE).

(81) Designated States (national): JP, US.

(84) Designated States (regional): European patent (AT, BE,  
CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC,  
NL, PT, SE, TR).

Published:  
— with international search report

(88) Date of publication of the international search report:  
25 April 2002

*For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.*

(54) Title: THERMOSTABLE CHIMERIC NUCLEIC ACID POLYMERASES AND USES THEREOF

(57) Abstract: Novel thermostable chimeric nucleic acid polymerases and methods for their generation and use are disclosed. It is shown that these chimeric nucleic acid polymerases, such as DNA polymerases, can be constructed using enzymatically active domains, isolated from different proteins or chemically synthesized. It is demonstrated that chimeric nucleic acid polymerases of the present invention possess the chemical and physical properties of their component domains (e.g., exonuclease activity, thermostability) and that the chimeric polymerases are thermostable.

WO 01/61015 A3

International Application No  
PCT/EP 01/01790

### A. CLASSIFICATION OF SUBJECT MATTER

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7	C12N15/54	C12N15/62	C12N15/70	C12N9/12	C12N1/21
	C1201/68	C12P19/34			

According to International Patent Classification (IPC) or to both national classification and IPC

### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12O C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, CAB Data, SEQUENCE SEARCH, BIOSIS, EPO-Internal

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
------------	--	-----------------------

X	WO 99 47649 A (ANKENBAUER WALTRAUD ;SCHOMBURG DIETMAR (DE); VILLBRANDT BRITTA (DE) 23 September 1999 (1999-09-23) the whole document	1-10, 36-43, 69-81
X	EP 0 892 058 A (HOFFMANN LA ROCHE) 20 January 1999 (1999-01-20)  the whole document	1-9, 36-42, 69-81
X	WO 97 29209 A (HARVARD COLLEGE) 14 August 1997 (1997-08-14)  the whole document	1,2,6-8, 36,37, 39-41, 69-81

- / - -

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

3 October 2001

Date of mailing of the international search report

18. 12. 2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer \_\_\_\_\_

HORNIG H.

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 01/01790

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 06200 A (CETUS CORP) 16 April 1992 (1992-04-16)  page 27, line 9 -page 28, line 7; claim 16 ---	1-10, 36-43, 69-81
P,X	GB 2 344 591 A (BIOLINE LIMITED) 14 June 2000 (2000-06-14)  claims 1-23 ---	1-11, 36-44, 69-81
E	WO 01 18213 A (DZIEGLEWSKA HANNA ;KRISTENSEN TOM (NO)) 15 March 2001 (2001-03-15) claims 1-20 -----	1-11, 36-44, 69-81



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP 01/01790

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

11, 44 completely; (1-10, 36-43, 69-81 partly)

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: (11,44)-completely, (1-10,36-43,69-81)-partially

A chimeric nucleic acid polymerase comprising at least two enzymatically active domains, wherein at least two of said domains are non-naturally associated, and said chimeric nucleic acid polymerase is thermostable; said polymerase comprises a 3'-5' exonuclease domain and a 5'-3' polymerase domain; said polymerase domain selected from Taq DNA polymerase comprising a Stoffel fragment of Taq polymerase or a mutant, variant, or derivative thereof; an isolated polynucleotide encoding said thermostable chimeric nucleic acid polymerase; a vector comprising said nucleic acid; a recombinant host cell transformed with said vector; a kit for polymerization comprising said chimeric nucleic acid polymerase; a process for nucleic acid polymerization using said chimeric polymerase; a process for producing said thermostable chimeric nucleic acid polymerase;

2. Claims: (12,24-30,45,57-63)-completely, (1-10,35-43, 68-81)-partially

Idem as invention 1 but limited to a 5'-3' polymerase domain of Taq DNA polymerase comprising amino acid residues 281-832 (SEQ ID No. 1); and wherein the chimeric polymerase are selected from: (i) 5'-3' polymerase domain from amino acid residues 281 to 832 of Taq DNA polymerase (SEQ ID No. 1), and 3'-5' exonuclease domain comprising amino acid residues 1-396 of Pho DNA polymerase (SEQ ID No. 3); (ii) 5'-3' polymerase domain from amino acid residues 281 to 832 of Taq DNA polymerase (SEQ ID No. 1), and 3'-5' exonuclease domain comprising amino acid residues 1-396 and/or residues 1-421 of Pwo DNA polymerase (SEQ ID Nos. 4 or 5); (iii) 5'-3' polymerase domain from amino acid residues 281 to 832 of Taq DNA polymerase (SEQ ID No. 1), and 3'-5' exonuclease domain comprises amino acid residues 1-508 of Sso DNA polymerase (SEQ ID No. 6) resulting in SEQ ID No. 10;

3. Claims: (13,31,32,46,64,65)-completely, (1-10,35-43, 68-81)-partially

Idem as invention 1 but limited to a 5'-3' polymerase domain of Taq DNA polymerase comprising amino acid residues 271-832 (SEQ ID No. 7); and wherein the chimeric polymerase are selected from: (i) 5'-3' polymerase domain from amino acid residues 271 to 832 of Taq DNA polymerase (SEQ ID No. 7), and 3'-5' exonuclease domain comprises amino acid residues 1-396 of Pho DNA polymerase (SEQ ID No. 3) resulting in SEQ ID No.8; (ii) 5'-3' polymerase domain from amino acid

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

residues 271 to 832 of Taq DNA polymerase (SEQ ID No. 7), and 3'-5' exonuclease domain comprises amino acid residues 1-396 of Pwo DNA polymerase (SEQ ID No. 4) resulting in SEQ ID No.9; (iii) 5'-3' polymerase domain from amino acid residues 271 to 832 of Taq DNA polymerase (SEQ ID No. 7), and 3'-5' exonuclease domain comprises amino acid residues 1-395 of Tpac DNA polymerase (SEQ ID No. 16) resulting in SEQ ID No.17; (iv) 5'-3' polymerase domain from amino acid residues 271 to 832 of Taq DNA polymerase (SEQ ID No. 7), and 3'-5' exonuclease domain comprises amino acid residues 1-421 of Pwo DNA polymerase (SEQ ID No. 5) resulting in SEQ ID No.11;

4. Claims: (14,33,34,47,66,67)-completely, (1-10,35-43, 68-81)-partially

Idem as invention 1 but limited to a 5'-3' polymerase domain of Tth DNA polymerase comprising amino acid residues 273-834 (SEQ ID No. 2); and wherein the chimeric polymerase are selected from: (i) 5'-3' polymerase domain from amino acid residues 273 to 834 of Tth DNA polymerase (SEQ ID No. 2), and 3'-5' exonuclease domain comprises amino acid residues 1-396 of Pho DNA polymerase (SEQ ID No. 3) resulting in SEQ ID No.12;

5. Claims: (15,16,48,49)-completely, (1-9,36-43, 69-81)-partially

Idem as invention 1 but limited to a 3'-5' exonuclease from *Pyrococcus horikoshii* Pho DNA polymerase, and SEQ ID No. 3; and insofar as not covered by the other group of inventions;

6. Claims: (17-19,50-52)-completely, (1-9,35,36-43, 69-81)-partially

Idem as invention 1 but limited to a 3'-5' exonuclease from *Pyrococcus woesei* Pwo DNA polymerase, and SEQ ID No. 4 and 5 and insofar as not covered by the other group of inventions;

7. Claims: (20,21,53,54)-completely, (1-9,35,36-43, 69-81)-partially

Idem as invention 1 but limited to a 3'-5' exonuclease from *Sulfolobus solfataricus* Sso DNA polymerase, and SEQ ID No. 6; and insofar as not covered by the other group of inventions;

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

8. Claims: (22,23,55,56)-completely, (1-9,35,36-43,  
69-81)-partially

Idem as invention 1 but limited to a 3'-5' exonuclease from  
Thermococcus pacificus Tpac DNA polymerase, and SEQ ID No.  
16; and insofar as not covered by the other group of  
inventions;

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 01/01790

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9947649	A	23-09-1999	DE 19810879 A1	16-09-1999
			WO 9947649 A2	23-09-1999
			EP 1112351 A2	04-07-2001
EP 0892058	A	20-01-1999	CA 2240570 A1	09-01-1999
			EP 0892058 A2	20-01-1999
			JP 11089588 A	06-04-1999
			US 6228628 B1	08-05-2001
WO 9729209	A	14-08-1997	US 5972603 A	26-10-1999
			WO 9729209 A1	14-08-1997
WO 9206200	A	16-04-1992	AT 181106 T	15-06-1999
			AU 691374 B2	14-05-1998
			AU 4086896 A	26-04-1996
			AU 663474 B2	12-10-1995
			AU 8668891 A	28-04-1992
			CA 2090614 A1	29-03-1992
			DE 69131321 D1	15-07-1999
			DE 69131321 T2	16-12-1999
			DK 550687 T3	13-12-1999
			EP 0550687 A1	14-07-1993
			EP 0894860 A1	03-02-1999
			ES 2134198 T3	01-10-1999
			GR 3031152 T3	31-12-1999
			JP 3227101 B2	12-11-2001
			JP 10004985 A	13-01-1998
			JP 2886842 B2	26-04-1999
			JP 10004965 A	13-01-1998
			JP 3227102 B2	12-11-2001
			JP 10000095 A	06-01-1998
			JP 2709311 B2	04-02-1998
			WO 9206200 A1	16-04-1992
			US 5405774 A	11-04-1995
			US 5795762 A	18-08-1998
			US 5466591 A	14-11-1995
			US 5455170 A	03-10-1995
			US 5674738 A	07-10-1997
			AT 197718 T	15-12-2000
			AU 658378 B2	13-04-1995
			AU 8907791 A	28-04-1992
			CA 2092317 A1	29-03-1992
			DE 69132477 D1	28-12-2000
			DE 69132477 T2	13-06-2001
			DK 550696 T3	19-03-2001
			EP 0550696 A1	14-07-1993
			ES 2152923 T3	16-02-2001
			JP 2582980 B2	19-02-1997
			JP 6504196 T	19-05-1994
			WO 9206202 A1	16-04-1992
			US 5968799 A	19-10-1999
GB 2344591	A	14-06-2000	NONE	
WO 0118213	A	15-03-2001	AU 7027500 A	10-04-2001
			WO 0118213 A1	15-03-2001